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Supercritical fluid extraction of bioactive compounds from berry seeds

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Abstract

Industrial processing of berries into food and beverages generates seeds as a by-product. Most of these seeds are treated as waste even though they contain valuable biological compounds. An approach to adding value to the process is to extract these compounds from the seeds.

The aim of this study was to investigate the yield and chemical composition of oils obtained by supercritical fluid extraction (SFE) from cloudberry, black currant, and bilberry seeds. The extractions were carried out at different pressures (200–500 bar) and temperatures (40°–80°C). Hexane extraction was performed as a reference.

The berry seed matrices had significant impacts on the extraction efficiency. As black currant seeds have small cells, the milling step resulted in smaller particles with larger surface area, thereby favouring the extraction. The increase in pressure at the same temperature enhanced the supercritical carbon dioxide (SC-CO_2) density, resulting in higher extraction yields. Increasing the temperature at 350 bar resulted in the higher levels of vitamin E and carotenoids in the recovered cloudberry and bilberry seed oils. The opposite effect was observed for black currants, due to differences in the seed matrix. The fatty acid composition of black currant seed oil, which includes stearidonic and γ-linolenic acid, suggests that this oil is a good source of polyunsaturated fatty acid. The low ω6/ω3 ratios (1.0–1.3) of cloudberry and black currant seed oils indicate that these oils could be interesting alternatives to fish oil supplements. In addition, the high levels of vitamin E seen in these oils suggest protection against lipid oxidation.

Keywords: Supercritical CO_2 extraction; Anti-oxidative activity; Berry seed oils; Fatty acids; Tocopherols; Tocotrienols; Carotenoids; Microscopy.

List of publications

This thesis is based on the studies described in the following papers, which are referred to in the text by their Roman numerals:

Paper I Graziele Gustinelli, Lovisa Eliasson, Cecilia Svelander, Marie Alminger and Lilia Ahrné. **Supercritical CO_2 extraction of bilberry (*Vaccinium myrtillus* L.) seed oil: composition and antioxidant activity.** The Journal of Supercritical Fluids, 2018, **135:** p. 91-97.

Paper II Graziele Gustinelli, Lovisa Eliasson, Cecilia Svelander, Thomas Andlid, Leif Lundin, Lilia Ahrné and Marie Alminger. **Supercritical fluid extraction of berry seeds: chemical composition, and antioxidant activity.** Submitted.

Contributions of the thesis author to the listed manuscripts:

Paper I Performed the experimental work and was responsible for writing the manuscript.

Paper II Performed the experimental work and was responsible for writing the manuscript.

Abbreviations

BHT	Butylated hydroxytoluene
CO_2	Carbon dioxide
CVD	Cardiovascular disease
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC_{50}	The concentration at which an extract reduces the DPPH absorbance by 50%
FA	Fatty acid
FAME	Fatty acid methyl ester
GC	Gas chromatography
HLPC	High-performance liquid chromatography
LM	Light microscope
MeOH	Methanol
MS	Mass spectrometry
MTBE	Methyl tert-butyl ether
MUFA	Monounsaturated fatty acid
ORAC	Oxygen radical absorbance capacity
TEAC	Trolox equivalent antioxidant capacity
FRAP	Ferric reducing antioxidant power
PUFA	Polyunsaturated fatty acid
SC-CO_2	Supercritical carbon dioxide
SCF	Supercritical fluid
SFE	Supercritical fluid extraction
T	Tocopherol
TAG	Triacylglycerols
Tr	Tocotrienol

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1 Introduction

The side-stream products generated by the food processing industry is mostly underutilised, being either incinerated or deposited in landfills, with consequent impacts on the environment [1]. The shortage of resources and increased demand for food underline the importance of using the side-streams from the food industry [2]. The utilisation of by-products, i.e., the valorisation of side-streams, confers economic and environmental benefits, such as reduced waste handling and landfilling, as well as the production of value-added products [3].

The annual production of wild berries in Scandinavia is 1 billion kilograms [4]. Berries (wild and cultivated) are commonly used as raw materials in food and beverages, thereby generating substantial side-streams in the forms of peel, pulp, and seeds [5, 6]. The juicing process removes the liquid (juice), leaving a paste that contains the peel and seeds [7]. Berry seeds, which are plentiful in Sweden, contain high contents of oil (≤30%), which can be extracted and used for food purposes. However, to maximise the value of berry seed by-products, the extraction process needs to preserve the biological compounds and their activities, while exerting a minimal environmental impact.

In this study, cloudberry, black currant, and bilberry seed oils were purified by supercritical fluid extraction (SFE) at different pressures (range, 200–500 bar) and temperatures (range,40°–80°C), to investigate the effects of pressure and temperature on the yields and chemical compositions of the obtained oils.

2 Objectives

The overall objective of this thesis work was to investigate the effects of pressure, temperature, solutes, and the structures of the seed matrices on the recovery of valuable compounds from cloudberry, black currant, and bilberry seeds extracted using SFE.

To achieve the overall objective, the following specific questions were addressed:

- Do differences in cell size and seed particle characteristics of the various berry seeds influence the extractability of the compounds?
- How do pressure and temperature influence the extraction yield and the recovery of antioxidants during SFE?
- What concentrations of fatty acids, vitamin E, and carotenoids are present in berry seed oils?

3 Background

3.1 Valorisation of fruit by-products from the food industry

The food industry is the second largest generator of waste, and has a deleterious impact on the environment that is second only to household sewage [8]. Over the last decade in Europe, 2.5×10^8 tonnes of side-stream products were generated annually from food processing [9]. The handling of this vast amount of side-stream entails economic and environmental problems, since it is usually burned or deposited in landfills [1, 10]. National laws have been changed to regulate waste management and promote sustainable development. In addition, the global shortage of resources and the increased demand for food, feedstuffs, and biofuel production require better application of resources to reduce waste generation and use the by-products [2, 6].

The by-products from the food industry include skin, peel, pomace, stones, stems, kernels, leaves, and seeds, which are sources of bioactive compounds, such as vitamins C and E, phenolic compounds, carotenoids, and fibrous biomass [8, 11]. There are several alternative ways in which by-products from the food industry can be used to reduce the need for side-stream disposal. The first alternative is the extraction of oilseeds, antioxidants (e.g., phenolic compounds, vitamin E, and carotenoids), and colorants (e.g., β-carotene)[12]. After this extraction, the dietary fibre and proteins can be recovered. The remaining biomass from the food industry can be used for the production of biofuels (e.g., biogas) and for composting [2, 12]. In addition, by-products can be used for animal feed. Currently, there are several examples of by-product utilisation: extraction of lycopene and β-carotene from tomato peels [13]; extraction of grape seed oil [14]; addition of citrus pulp to pig feed [15]; use of citrus and apple pomace for the production of dietary fibre powders [12]; and production of biogas from cocoa residues [16]. However, information on the utilisation of by-products from berries remains scarce.

3.1.1 High valuable compounds in berries

Berries are composed of major constituents (sugars, polysaccharides and lipids from different classes) and minor constituents (e.g., phenolic compounds, vitamin E, and carotenoids). Berries are a source of dietary fibres (1–7% f.w.), which are cell wall polysaccharides, being composed of pectin, lignin, hemicellulose, and cellulose [17, 18]. A major part of the dietary fibre that remains after the extraction process is insoluble, although it becomes solubilised after enzyme treatment [19]. Phenolic compounds are secondary metabolites in plants and have antioxidant activities. In berries, the most common forms being flavonols and anthocyanins, with the latter being responsible for the dark colour in black currants and bilberries [20].

Berry seeds are rich in triacylglycerols (TAG), and the predominant fatty acids (FAs) are palmitic, oleic, linoleic, and α-linolenic acids [21]. Linoleic and α-linolenic acids represent together 60 to 80% of the total FA of berry seed oils [5]. Moreover, they are essential FAs, be metabolized to arachidonic and eicosapentaenoic acids, respectively [22]. Phospholipids, which are amphiphilic molecules that make up the bilayer membranes of plant cells, are often associated with fatty acids and vitamin E [19, 23]. Berry seed oils are also rich in minor compounds with antioxidant properties such as vitamin E and carotenoids [24]. Tocols, or vitamin E, are powerful antioxidants that protect the PUFAs, acting as peroxyl radical scavengers by donating hydrogen atoms [25]. Vitamin E is mainly synthesized in chloroplast membranes and oil bodies of seeds, and can be found in significant amounts in berry seed oils (400 mg/100 g of raspberry seed oil) [5]. Carotenoids is a group of naturally occurring pigments comprising about 750 compounds [26]. These compounds may also have antioxidant activity, by quenching of electronically excited molecules such as singlet oxygen [27]. The health benefits of vitamin E and carotenoids and the increasing demand for natural products has increased the interest in extract these compounds [28]. However, vitamin E and carotenoids are easily oxidized and therefore, it is important to avoid exposing these compounds to oxidation during sample preparation, extraction and storage [29, 30]. The mild temperature and absence of air and light during SFE make this extraction method suitable to preserve thermo-labile compounds [31]. The high temperature used in some conventional

extractions and the refining steps can damage the bioactive compounds [32].

3.1.2 Determination of biological compounds and their bioactivity

The chemical diversity of antioxidants can hinder the separation, detection and quantification of individual antioxidants from food [33]. Therefore, different chromatographic methods have been developed to characterize and quantify the antioxidant compounds. Prior the analyses, an extraction step should be performed to isolate the compound of interest from the matrix [34]. The fatty acid composition can be determined and quantified by gas chromatography (GC) [35]. The TAGs are normally extracted by the Bligh and Dyer method based on chloroform and methanol [36]. The extracted samples are then subjected to transesterification, followed by methylation [37]. The fatty acid methyl esters (FAMEs) are analysed by GC. To determination of vitamin E and carotenoids, the samples are also subjected to an extraction step, to isolate the compounds to be analysed. The separation of vitamin E and carotenoids are done by the high-performance liquid chromatography (HPLC), where the compounds can remain stable [26, 30]. The antioxidant activity and capacity can be assessed by spectrophotometric by hydrogen atom transfer-based assay, such as oxygen radical absorbance capacity (ORAC) or electron transfer-based assays, such as trolox equivalent, antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) [33]. The measurement principle of TEAC, FRAP and DPPH is absorbance while of ORAC is fluorescence [33]. The DPPH assay is commonly used due to its ease of use and low cost [33]. Moreover, the DPPH method is suitable for evaluating the free radical scavenging activities of compounds, such as vitamin E.

3.1.3 Berry seeds as by-products

Berries are widely used as raw material in the food industry, e.g. for processing of juice, resulting in large volumes of side-streams that can be used as a source for extraction of bioactive compounds. Part of the side-streams can be obtained s during the pre-treatment phase, with washing and sorting of the berries, and the separation of damaged fruits, stems, and stalks from the remaining material [6]. After the pre-treatment stage, the berries are often pressed into juice,

generating large amounts of press cake [18]. The press cake from berry juice production contains seeds with up to 30% oil (dry weight, d.w.) [21]. The high oil content of berry seeds makes these by-products an attractive source of oil.

3.2 Extraction of valuable compounds

As extraction is the first step in studying the biological compounds from a plant, the extraction method should be selected carefully [34]. Soxhlet-mediated solvent extraction and cold pressing are traditionally used technologies for extracting oils from seeds. In contrast, SFE is a non-conventional method of extraction which can be used as alternative to replace conventional methods.

Phenolic compounds such as anthocyanins can be extracted with polar compounds due to having a low molecular weight [34]. The extraction of anthocyanins is usually performed with water and organic solvents such as methanol and ethanol [34, 38]. These solvents can also be used as co-solvent in SFE to expand the extraction range and hence, to extract phenolic compounds [32]. FAs are non-polar molecules, which means that they are soluble in non-polar solvents. Lipid antioxidants, such as vitamin E and carotenoids, are also soluble in non-polar solvents, such as hexane or SC-CO_2. However, some carotenoids can form crystals, which reduces their solubility [28].

3.2.1 Conventional solvent extraction

Soxhlet-mediated solvent extraction takes several hours to perform and uses non-selective organic solvents, which require a refining step [32, 39]. Soxhlet extraction uses an apparatus that performs the extraction in two steps: boiling (at the solvent boiling point) and rinsing, followed by recovery of the extract [40]. Solvent extraction immerses the sample in solvent, at a solvent:sample ratio of 5–10:1, and the solutes are extracted from the matrix while they are mixing on a rotary plate [41].

Hexane and ethanol are organic solvents that are commonly used in the Soxhlet and solvent extractions. The polarities (dipole moments) of hexane and ethanol are respectively 0.0 and 5.2 [42]. As the linkages between ethanol molecules are hydrogen bonds, ethanol is miscible in water. The boiling point of ethanol (78.37°C) is higher than that of hexane (68°C), and in

the Soxhlet extraction, which operates at the boiling point of the solvent, ethanol can be more deleterious for thermo-labile compounds. Solvent extraction performs at room temperature and the solvent removal is carried out at ≥40°C; however, the solvation power is weaker compared to Soxhlet extraction. In general, Soxhlet and solvent extraction using hexane often results in higher extraction yields compared to non-conventional methods, due to the low polarity of hexane [43].

3.2.2 Supercritical fluid extraction: theory and applications

SFE is a non-conventional method of extraction which uses supercritical carbon dioxide (SC-CO_2) as solvent, which is non-toxic, non-flammable, can easily be removed from the extracts, and possesses liquid-like densities and gas-like viscosity [44-46]. The parameters of extraction, such as pressure, temperature, extraction time, pre-treatments, and matrix features, all influence the extraction yield and oil quality. The solubility levels of compounds are directly affected by the pressure and temperature settings during extraction. Pressure is the main parameter in SFE, and in general, an increase in pressure leads to an increase in the supercritical fluid (SCF) density, thereby increasing the solubility. SFE using SC-CO_2 as solvent can operate under mild temperature conditions (≥31.1°C), thereby preserving thermo-labile compounds [32]. The influence of temperature on the SFE depends on the magnitudes of two phenomena: when the temperature increases, the SC-CO_2 density decreases and the vapour pressure of the solutes increases [47]. Previous studies have reported a positive effect on extraction yield in association with an increase in temperature at the same pressure [48, 49].

Sample pre-treatment protocols modify the physicochemical characteristics of the raw materials and can be adapted to improve the extraction efficiency [50]. The drying step damages the cell walls, facilitating the extraction of compounds and reducing the moisture content, which at high levels can compete with SC-CO_2 as the solvent [32, 51]. The heat used for drying can also break the bonds between the carotenoids and the matrix, favouring the release of the former during extraction [50]. Milling disrupts the cell walls, thereby increasing the surface area and the contact between the solvent and matrix [52]. Moreover, this process

reduces the diffusion path in the solid matrix, which decreases the mass transfer resistance to solute diffusion [51]. Increases in extraction yield associated with longer milling times or smaller particle sizes have been reported in the literature [45, 49, 53]. Pre-treatment with enzymes (e.g., cellulases and proteases) hydrolyses the structural polysaccharides, which form the cell and lipid body membranes [14, 50, 54]. Consequently, the cellular and subcellular structures are partially destroyed, decreasing the mass transfer resistance, which facilitates oil release and increases the extraction yield [55]. Previous studies [14, 50, 54] have reported increased extraction yields from plant matrices that were pre-treated with enzymes and subjected to SFE or cold-pressing extraction. Even though several studies have investigated pre-treatment protocols for different matrices, information on the pre-treatment of berry seeds is scarce.

Solubilities of compounds from berry matrices

The solubility of a compound in a specific solvent depends on the polarity and molecular affinity between the solvent and solute [34]. Chrastil [56] developed an equation, based on the association laws and/or the entropies of the components, that establishes the general trends of solubility behaviour. However, oils are complex mixtures of FAs, mono-, di-, and tri-acylglycerols, and minor compounds. Therefore, the solubility behaviour of oil in SCF is more complex than a simple binary system and depends on intermolecular interactions [28]. In systems in which the solute and solvent have similar bonds, the freedom of movement of the solute in the solvent is approximately equivalent to their initial levels of freedom of movement, and the solute can solubilise in the solvent. When their bonds are different, the respective bonds need to be broken and new bonds will be formed between solute and solvent, resulting in changes in the structure of the solvent, which needs to compensate for the loss of the previous bonds and the formation of weaker solute-solvent attractions [57]. The molecules in this new arrangement have lower entropies and therefore, are less dispersed than before. According to the Second Law of Thermodynamics, entropy always tends to increase, so a less-dispersed arrangement is less likely to exist [57, 58].

Properties of solvents

Ethanol or water can be added to SFE as a co-solvent to increase the polarity and to solubilise polar compounds (e.g., phenolic compounds) through the formation of hydrogen bonds [59]. The solubility levels of the compounds in the SCF solvent depend on the pressure and temperature, and minor adjustments to the pressure and temperature alter the density of the SC-CO_2, and thereby, the solubilities of the compounds contained in this solvent. This offers the potential to fractionate compounds based on the solubilities of targeted compounds [28]. The SCFs have liquid-like densities and gas-like viscosities, and their diffusivity values are about two orders of magnitude higher than those of typical liquids [44]. These unique properties allow SCFs to penetrate porous materials with less resistance than a liquid solvent and with more solvation power [59].

SC-CO_2 operates under mild conditions, since its critical temperature is above 31.1°C and critical pressure is above 73.8 bar [32]. According to Saharay and Balasubramanian [60], CO_2 is a linear molecule with a bond angle of 180°C. In the supercritical state, CO_2 is marginally non-linear with a bond angle of 174.2°C. Thus, CO_2 possesses a dipole moment, which in the supercritical fluid state changes its internal geometry to a configuration with pairs of molecules oriented in a distorted T-shaped geometry. This arrangement is believed to possess a quadrupole moment, which enables the solvation of molecules, such as aldehydes, alcohols and esters, through electron donor/acceptor interactions with the CO_2 molecule, which explains the solvation power of SC-CO_2 [59]. Solutes tend to reside in regions of high solvent density, which are enhanced by quadrupolar effects, leading to the clustering of SCF around the solute [61].

Effects of matrix on SFE

The targeted compounds can be found at various levels in different parts of the same plant. Plant leaves are rich in chloroplasts, which generate reactive oxygen species and have high levels of vitamin E, especially α-tocopherol, due to its ability to scavenge singlet oxygen; in plant seeds, the vitamin E is mostly located in the membranes of cytoplasmic

lipid bodies [62, 63]. The levels of carotenoids also vary between different parts of plants. Some carotenoids accumulate in green leaves, reproductive tissues, petals, and fruits, including seeds [64]. The locations of solutes inside the matrix can also influence the extraction efficiency, since mass transfer resistance is directly dependent upon the matrix structure. Seed cells from berries have thick cell walls [17], which can increase the mass transfer resistance, and they probably need longer milling times to make the solutes available for extraction.

Figure 1 shows a schematic of a plant cell. The lipids are stored in organelles called 'oil bodies', which contain TAG,

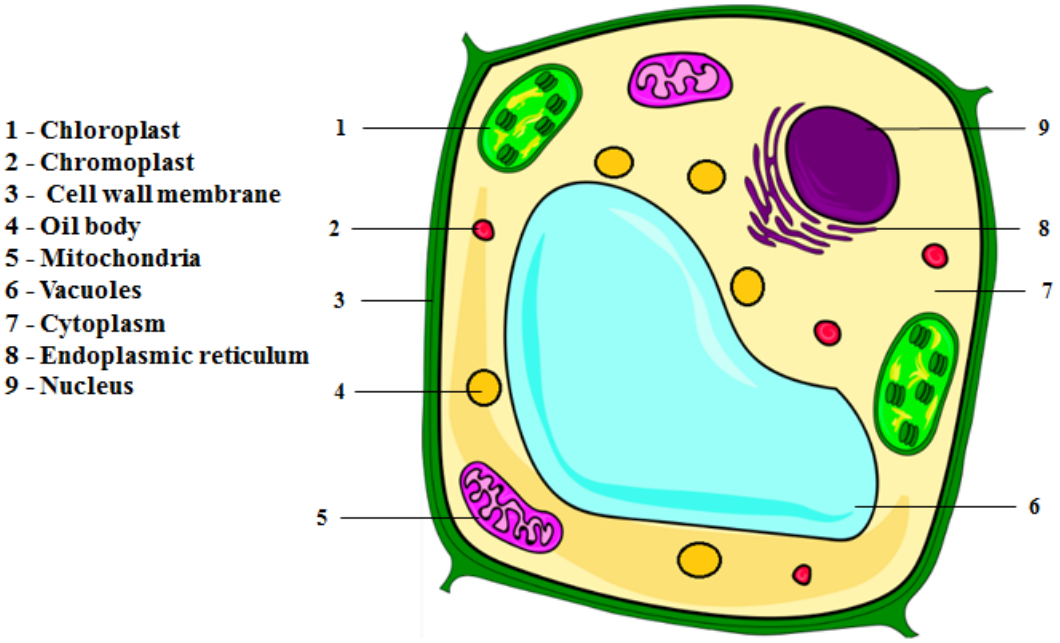

Fig. 1. Schematic illustration of plant cell shows its main structures. The image was kindly provided by Ana Chung.

intrinsic proteins, phospholipids, and bioactive compounds [65]. The oil bodies are developed during seed maturation from the endoplasmic reticulum in embryonic cells [66]. The lipids from oil bodies become available for extraction when the cell walls (which are impervious to extraction solvents) are disrupted in a pre-treatment process. **Figure 2** shows a schematic overview of the four stages inside a matrix during SFE. As described previously [45, 67, 68], in the initial stage (**1**), the SCF diffuses into the matrix, enabling the swelling of cellular structures due to the absorption of SCF. Then (**2**), the solute dissolves in the SCF. While the solute strongly interacts with both the SFC and matrix, the SCF is inert for the cell wall. The mixture (SCF plus solute) is

transferred by diffusion (**3**) to the outer surface of the matrix: Finally, (**4**) the mixture flows away from the matrix to the extractor vessel, where the solute can be collected.

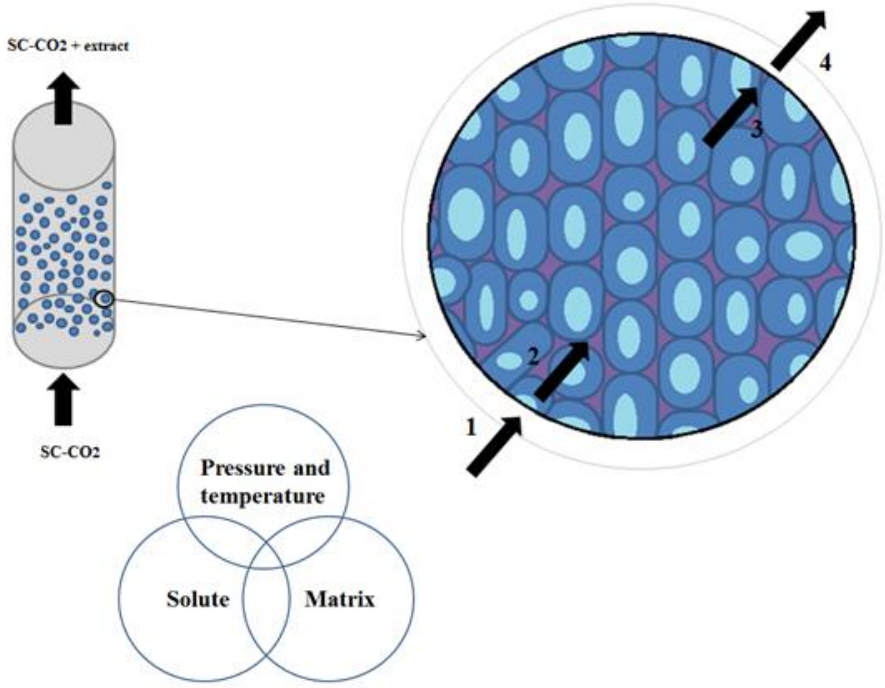

Fig. 2. Schematic overview of SFE: (1) supercritical fluid diffuses into the matrix; (2) the solute dissolves in the supercritical fluid; (3) the mixture (extracted substance and supercritical fluid) diffuses to the outer surface of the matrix; and (4) the mixture flows away from the matrix. The rings illustrate the main aspects involved during SFE and the connection between each other.

Experimental design

The experimental design is a statistical strategy to achieve high extraction efficiency with a minimal number of experimental runs [69]. The experimental designs for SFE can be divided in screening and optimization design [70]. The screening designs comprise the factorial designs, which can evaluate at least two factors at two or more levels [70]. **Figure 3** presents a two-level factorial design, characterized by two number of factors (pressure and temperature), at two different levels with a mid-point. The optimization designs determine the optimal conditions with a smaller number of extractions and they often start with a screening design to determine the most important factors [70]. The most used optimization designs are central composite design, box-Behnken design and response surface methodology [69-71].

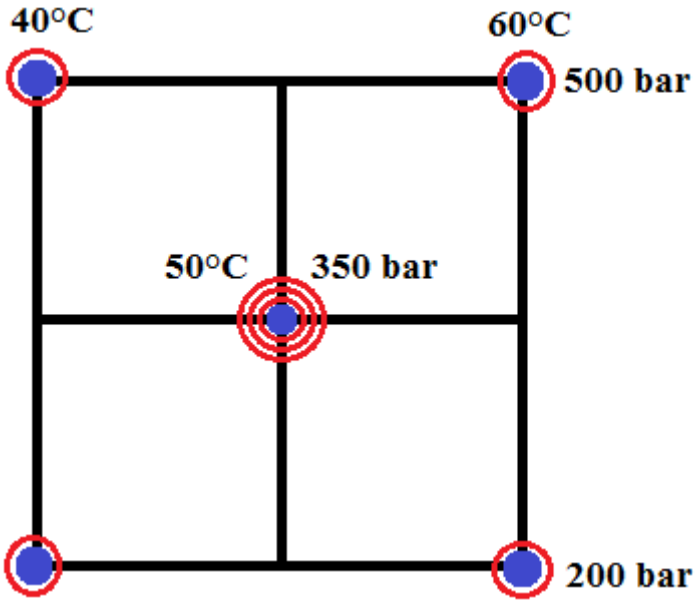

Fig. 3. Experimental design for bilberry seed extractions. Five extraction conditions were studied. The mid-point, 300 bar and 50°C was repeated 3 times. The corner points were, 200 bar and 40°C; 200 bar and 60°C; 500 bar and 40°C; and 500 bar and 60°C.

4 Materials and methods

This chapter presents an overview of the studies included in this thesis and describes the materials and methods used in this work, including: the raw materials used; the pre-treatment protocols applied prior to the extractions; the methods' development; the final method of analysis; and the statistical analysis.
The methods are written in details in the attached papers while in this section only the essential parts were described.

4.1 Study overview

SFE of berry seed oils was carried out under different conditions, to investigate the effects of pressure and temperature on the yields and chemical compositions of the extracted oils. **Figure 4** presents an overview of the study 1 and study 2. In the study 1, the chosen conditions for SFE were based on a two level full factorial design (**Figure 3**) and only bilberry seeds were used. In Study 2, cloudberry, black currant and bilberry seeds were used and only two conditions were selected. Conventional extraction was also carried out for comparison.

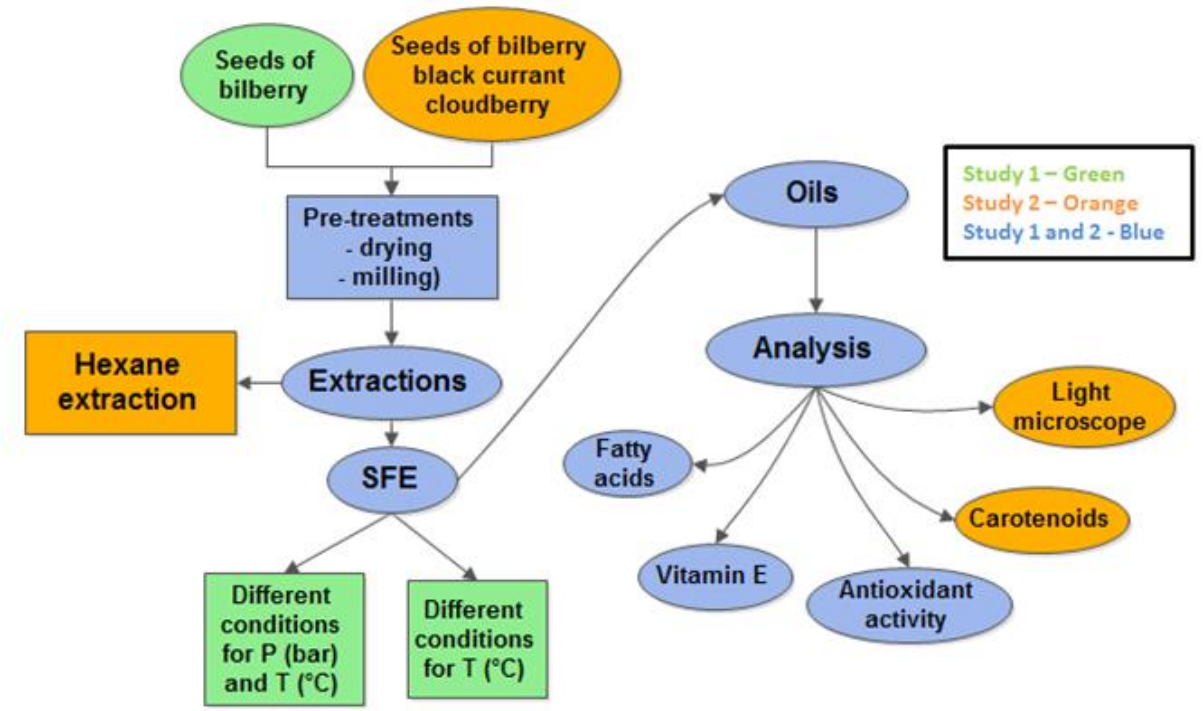

Fig. 4. Overview of the outline of the study. The different parts with raw material; pre-treatments of seeds; extraction conditions, and analysis are highlighted with different colours: study 1 in green, study 2 in orange and, study 1 and 2 in blue.

The berry seeds were dried and milled before each extraction. The factorial design presented in **Figure 3** was used for screening of processing conditions during the extraction of bilberry seed oil. The screening study showed that an increase in temperature, but at the same pressure, can have a positive effect on the recovery of bioactive compounds. The selected experimental conditions used in the subsequent extractions of cloudberry, black currant, and again, bilberry seed oils were based on the screening study. Chemical analyses were performed to determine the properties of the obtained oils. In addition, the microstructures of the dried and milled seeds were monitored using light microscopy, to elucidate the influence of cell morphology on the extraction efficacy.

4.2 Raw materials

All the berries used in this work were harvested during the berry season and processed into juice.

Wild bilberries (*Vaccinium myrtillus* L.) were harvested in Harads, Sweden. After the bilberries were processed into juice, the seeds were washed with water and left to precipitate. The seeds were reasonably clean and free of residues. These seeds were extracted under the conditions listed in **Figure 3**.

For the secondary trials, the berries were harvested in Norrbotten, Sweden. Bilberries (*Vaccinium myrtillus* L.) and cloudberries (*Rubus chamaemorus*) were wild-grown, while the black currants (*Ribes nigrum* L.) were cultivated. A pureeing machine was used to separate the bilberry and black currant seeds from the (initial) press cake. For whole cloudberries, the seeds were directly separated in the pureeing machine. The final seed batches still contained a small fraction of the press cake, which could not be totally eliminated from the seeds.

The cloudberry, black currant, and bilberry seeds were stored at -20°C until the extractions.

4.3 Sample preparation

The berry seeds were thawed for 6 h. After thawing, the seeds were dried at 40°C for 16 h. The black currant seeds became sticky and a crust formed on top of the seeds, probably due to their high content of sugar. This problem was solved in a subsequent trial by mixing the seeds regularly during the first hours to ensure uniform drying. The final moisture contents were: 2.7% for the bilberry seeds from the first batch; and 6.5% for each of the cloudberry, black currant, and bilberry seeds. The dried cloudberry, black currant, and bilberry seeds were stored at -20°C until the extractions.

The seeds were milled in a coffee grinder for 30 s just before each extraction. The particle size of the milled bilberry seeds (from the first batch) was determined using a sieve-shaker. The particle size distributions were: <250 µm (0.34%); 250-500 µm (6.08%); 500-710 µm (75.04%); and >710 µm (18.54%). The particles of the bilberry seeds tended to stick to the sieve mesh because of their high oil content. Moreover, the particles were heterogeneous in shape, making it difficult for some of them to pass through the mesh.

4.4 Conventional solvent extraction

Conventional solvent extraction was performed for the cloudberry, black currant, and bilberry seeds, to compare with SFE.

Milled seeds and hexane were mixed for 2 h and then centrifuged. The supernatants were transferred to another tube and the procedure was repeated. The supernatants were pooled and the solvent was evaporated by purging the samples with N_2 at 40°C. The evaporated extracts were stored at -18°C until analysed.

4.5 Supercritical fluid extraction

The SFE conditions for the bilberry seeds were chosen based on the factorial design presented in **Figure 3**. The corner conditions were chosen based on the variations of the densities of SC-CO_2 (**Table 1**). The mid-point extraction was performed in triplicate. Pre-trials with the chosen conditions were carried out to decide on the extraction time. Samples were collected every 10 min for a total of 110 min, to obtain an extraction curve for each condition. The extraction time

chosen was 80 min. The design was executed in duplicate, with two extractions performed for each corner condition (200 bar and 40°C; 200 bar and 60°C; 500 bar and 40°C; and 500 bar and 60°C) and six extractions performed for the mid-point (350 bar and 50°C). The flow rate was 40 g CO_2/min.

Table 1
Experimental conditions for supercritical CO_2 extraction of berry seeds, and density of SC-CO_2

Berry seed	Extraction conditions	Extraction time	Density (kg/m3)	Extraction yield (% w/w)
Bilberry	200 bar 40°C	80	840	19.5 ± 0.1
Bilberry	200 bar 60°C	80	724	7.6 ± 0.6
Bilberry	350 bar 50°C	80	899	21.8 ± 0.8
Bilberry	500 bar 40°C	80	991	22.0 ± 0.9
Bilberry	500 bar 60°C	80	934	22.2 ± 1.1
Cloudberry	350 bar 50°C	60	899	3.4 ± 0.2
Cloudberry	350 bar 80°C	60	789	6.7 ± 0.9
Black currant	350 bar 50°C	60	899	2.0 ± 0.1
Black currant	350 bar 80°C	60	789	6.1 ± 0.4
Bilberry	350 bar 50°C	60	899	13.2 ± 0.5
Bilberry	350 bar 80°C	60	789	12.4 ± 0.5
Cloudberry	Hexane extraction	240	659	10.4 ± 0.8
Black currant	Hexane extraction	240	659	10.0 ± 0.3
Bilberry	Hexane extraction	240	659	18.8 ± 0.4

The SFE conditions for the cloudberry, black currant and bilberry seeds were chosen based on the findings from Study 1. The conditions of 200 bar and 60°C and 350 bar and 50°C were selected. However, the yields of oils extracted at 200 bar and 60°C were too low, being economically unfeasible. The effect of temperature on the recovery of vitamin E in term of the variability in density (**Table 1**) was considered, so as to select another extraction condition. The eventual extraction conditions selected were 350 bar and 50°C and 80°C, and the extractions were done in triplicate. The extraction time was 60 min and the flow rate was 30 g CO_2/min.

4.6 Fatty acid analysis

The fatty acids from the berry seed oils were methylated as described on the method of Cavonius and co-workers [37]. After methylation, Milli-Q water was added, followed by petroleum ether. The solutions were vortexed and centrifuged, to separate the organic phase containing the FAMEs. The samples were analysed by gas chromatography (GC) with a triple-axis mass spectrometry (MS) detector in electron impact mode.

Quantification was performed with an internal standard (C 10:0), and the identification of the different peaks was accomplished using external standards.

4.7 Vitamin E analysis

The sample preparations needed to be optimised in order to separate the vitamin E from the other compounds present in the extract.

4.7.1 Development of sample preparation for vitamin E analysis

The sample preparation was developed based on experiments in which the aim was to dilute the sample (oil) or extract the vitamin E from the sample. The solvents tested were: 2-propanol, a mix of methanol and hexane (1:1), and methanol [5, 72]. In the first trial, the samples were diluted in 2-propanol; however, the peaks obtained in the HPLC chromatograms were very small. The second trial was designed to separate the vitamin E from the other compounds based on differences in polarity. Hexane has a lower degree of polarity than methanol, so it could be used to dilute the other compounds present in the oil while the vitamin E was diluted in methanol. The HPLC peaks were still smaller than expected, and the method showed low repeatability. In the third trial, the oils were mixed with 2 mL of methanol, vortexed, and sonicated for 15 min. The sonication diluted the vitamin E in the methanol while the other compounds moved to the bottom of the tube. The upper fraction was collected (1 mL) and analysed. The method showed consistent results, with high repeatability and better peak responses stronger HPLC peaks.

4.7.2 HPLC separation of vitamin E

Tocopherols and tocotrienols were separated using a reverse-phase C18 column. The peaks were detected using a fluorescence detector. The mobile phase was a mixture of methanol and water (95:5 v/v). The excitation and emission wavelengths were 295 and 330 nm, respectively. The peaks were identified using standards and quantified using calibration curves. The concentrations were confirmed using the extinction coefficient.

4.8 Carotenoid analysis

The analysis of carotenoids, as for the analysis of vitamin E, required optimisation of sample preparation. Given the unstable nature of carotenoids, the samples were protected from light and heat during all the steps.

4.8.1 Development of sample preparation for carotenoid analysis

Similar to the analytic system used for vitamin E, preparation of the sample was designed either to dilute the sample or to separate the carotenoids from the samples. The solvents tested were: acetone plus 0.1% butylated hydroxytoluene (BHT; used to protect the samples against lipid oxidation); a mixture of MeOH (methanol) and methyl tert-butyl ether (MTBE; 75:25); and MTBE. In the first trial, the samples were diluted in the solvent and incubated in the freezer at -20°C for 2–16 h, to separate the carotenoids from the oil, as recommended for samples with a high content of oils [73]. The HPLC signal intensities were lower than expected and the method showed low reproducibility. In the second trial, the samples were not properly diluted, resulting in low signal intensities. In the third trial, the samples were properly diluted and the results showed appropriate reproducibility. However, several of the HPLC peaks overlapped and the initial gradient had to be optimised. For the bilberry and black currant seed oils, the peaks were concentrated in the initial part of the chromatogram, corresponding to the xanthophylls, while the cloudberry seed oil had multiple peaks for carotenoids overlapping in the final part of the chromatogram.

4.8.2 HPLC separation of carotenoids

Carotenoids were separated using reverse-phase elution in a C30 column. The following gradient generation procedure was used for the black currant and bilberry seed oils: an isocratic elution of 85% (v/v) MeOH and 15% (v/v) MTBE was retained for 2 min; the gradient was established over a period of 9 min with a composition of 75% MeOH and 25% MTBE; for the next 12 min, the gradient comprised 10% MeOH and 90% MTBE; in the following 3 min, the gradient reached the initial composition of 85% MeOH and 15% MTBE, followed by isocratic

elution for final 4 min. For the cloudberry seed oil, the gradient construction was as follows: the initial condition of 85% MeOH and 15% MTBE was established over a period of 6 min to be 75% MeOH and 25% MTBE, and then to 60% MeOH and 40% MTBE over 1 min and 10% MeOH and 90% MTBE for 16 min; the gradient was reset to the initial conditions over a period of 3 min and then equilibrated for an additional 6 min.

The peaks were detected using a spectrophotometer that was equipped with a UV-visible photodiode array detector. The absorption spectra were scanned from 250 nm to 550 nm. The mobile phase consisted of methanol and MTBE. The peaks were identified using a spectral library, comparing the spectra and corresponding retention times with standards, and quantified using calibration curves created with external standards. The concentrations were confirmed using the extinction coefficient.

4.9 Antioxidant activity

The analysis of antioxidant activity also required optimisation of sample preparation. The DPPH solution was prepared in amber glass due to its sensitivity to light and the samples were protected from light and heat during all the steps.

4.9.1 Development of sample preparation for DPPH assay

The sample preparation procedure was developed based on several studies on the DPPH method [74-76]. The solvents that were tested to dilute the samples were: MeOH, acetone, and ethanol. As the oils were only partially soluble in MeOH, this solvent was considered unsuitable. While the oils were totally soluble in acetone, this solvent gave low values for the extent of reduction [77]. Finally, the oils were soluble in ethanol, which is a suitable solvent for the DPPH method. The samples for study 2 needed to be filtered in paper filter to remove the solid particles.

Different concentrations of oil were tested for the different berry seed oils to identify a range of concentrations that fit with the obtained values for the final absorbance. The absorbance values were measured in a spectrophotometer at 517 nm.

After mixing the oil solution and the DPPH solution, the absorbance was evaluated every 10 min, to determine when the reaction had reached the steady state. For Study 1, the steady state was reached after 40 min, while for Study 2 the reactions required 1 h to reach completion.

4.9.2 Free radical scavenging activity by DPPH assay

The samples were diluted to obtain five different concentrations for determination of the efficient concentration (EC_{50}). An aliquot of 1 mL of each dilution was mixed with 1 mL of a freshly prepared solution of DPPH (100 µM). Solutions of α-tocopherol were used as the positive control. The mixtures were incubated in the dark at room temperature for 40 min and 60 min for Study 1 and Study 2, respectively. The absorbance values were read at 517 nm in a spectrophotometer. The efficient concentration (EC_{50}) was calculated using linear regression analysis.

4.10 Light microscopy (LM) analysis

The microstructures of the milled seeds of cloudberry, black currant, and bilberry were investigated under LM. The seed particles were smeared on the surfaces of microscope slides and a droplet of water was added to disperse the seed fragments.

The cloudberry, black currant, and bilberry seed oils were also examined under LM. The oil droplets were poured into the wells of diagnostic microscope slides. Objective lenses giving magnification ×10 and ×20 were used for examinations of both the seeds and oils.

4.11 Statistical analysis

The statistical significance of differences between groups and the interaction between pressure and temperature in study 1 were tested using two-way ANOVA. Additionally, the interactions were explored using the software MODDE 11 from Umetrics (Kinnelon, NJ, USA). The statistical significance of differences between groups in study 2 was tested using ANOVA. The ANOVA tests were followed by a Tukey post-hoc test (level of significance, $p \leq 0.05$).

5 Results and Discussion

In this thesis, the effects of key parameters, such as pressure and temperature, on the yields and compositions of the extracts obtained from berry seed by-products are examined. The utilisation of berry seed oils obtained by SFE is discussed in relation to the health aspects, quality and stability of the berry seed oils, and the sustainability of using oils derived by SFE from by-products.

5.1 Parameters influencing extractability during SFE

Different aspects of the major parameters (pressure and temperature), solute, and matrix in relation to SFE are discussed in detail in this section.

5.1.1 Effect of pressure

The effects of pressure on the extraction efficiency of bilberry seeds were investigated using 200, 350 and 500 bar at 40°C, 50°C, and 60°C (**Fig. 4**). **Figure 5** presents the extraction curves used to choose the extraction time. The extraction yields (% w oil/w dry seeds) at 110 min were 6.3% and 22.6% at 200 bar and 60°C and at 500 bar and 60°C, respectively. The effect of increasing the pressure was more prominent in the initial (40-min) phase of the extraction, during which period the oil is rapidly extracted from the surface and sub-surface of the matrix, the mass transfer resistance is low, and the extraction efficiency is more dependent upon the oil solubility [14, 55]. An increase in pressure under isothermal conditions increases the density and enhances the solubility of the oil, thereby increasing the driving force of mass transfer [14]. The increased oil solubility at 500 bar (**Fig. 5**) may have resulted in the saturation of SC-CO_2, as indicated by the overlapping curves at 10 min and 20 min. The solubility of the oil in the SC-CO_2 until the mixture (oil + SC-CO_2) was released from the matrix is shown in Steps 2–4 in **Figure 2**. At 200 bar, the extraction yields were significantly lower than at 500 bar, indicating that the solubility of the extract in SC-CO_2 was enhanced at 500 bar.

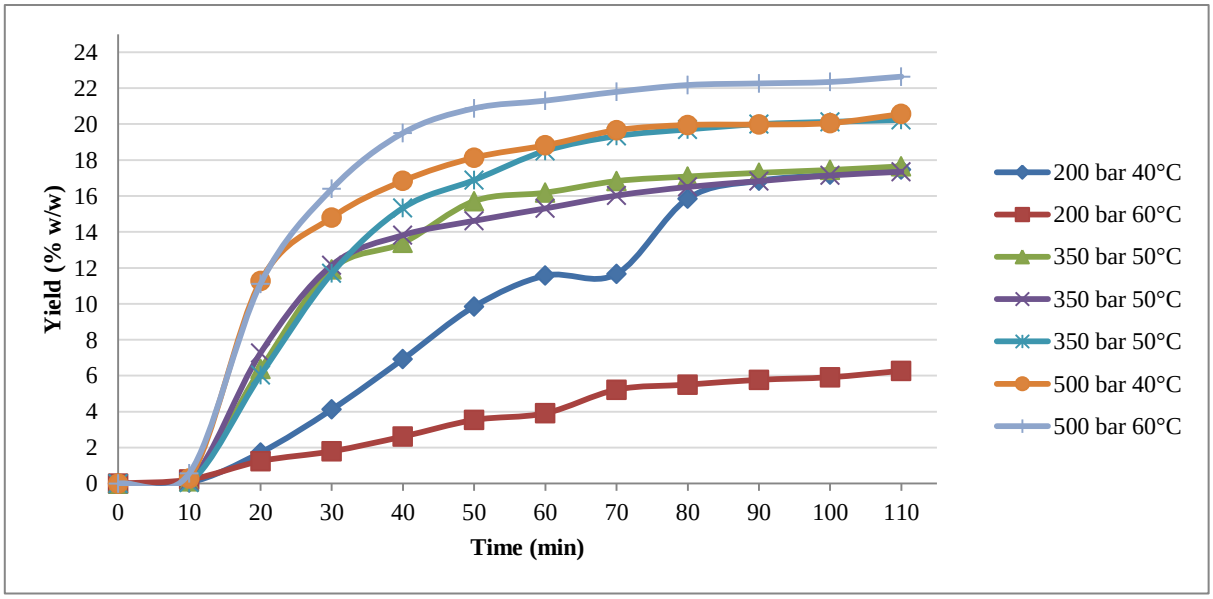

Fig. 5. Extraction curves for the SFE of bilberry seed oils extracted at different pressures and temperatures for 110 min.

The SFE process is manifested as two different extraction periods: the first period is characterised by rapid oil extraction due to fast extraction of the oil at the surface and shallow sub-surface, where the transport mechanism is convection; the second period is slower and controlled by diffusion of the supercritical fluid into the matrix [14, 55]. To ensure an efficient extraction process, it is important to define the transition that occurs between these periods. **Figure 6** shows the extraction rates (yield %/min) of the extraction curves. The first period of extraction occurred in the first 40 min and 50 min for the samples extracted at high pressures (350 bar and 500 bar) and at 200 bar, respectively. Thereafter, there was a transition to the second period of extraction, represented by the declining rate of extraction. At 80 min, the extraction rates were very low in most of the curves, and continuing the extraction for a longer period would not have been beneficial. SFE of bilberry seeds was repeated using the same conditions used in the extraction curve (**Fig. 5**), and the oils were collected at 80 min.

In **Figure 7**, it is clear that the different extraction conditions influenced the extraction yields. As in the case of the extraction curves (**Fig. 5**), the lowest yield was obtained at 200 bar and 60°C, and the highest yield was noted at 500 bar and 60°C. The increase in pressure (350 bar and 500 bar) led to a higher level of oil recovery, regardless of the temperature used.

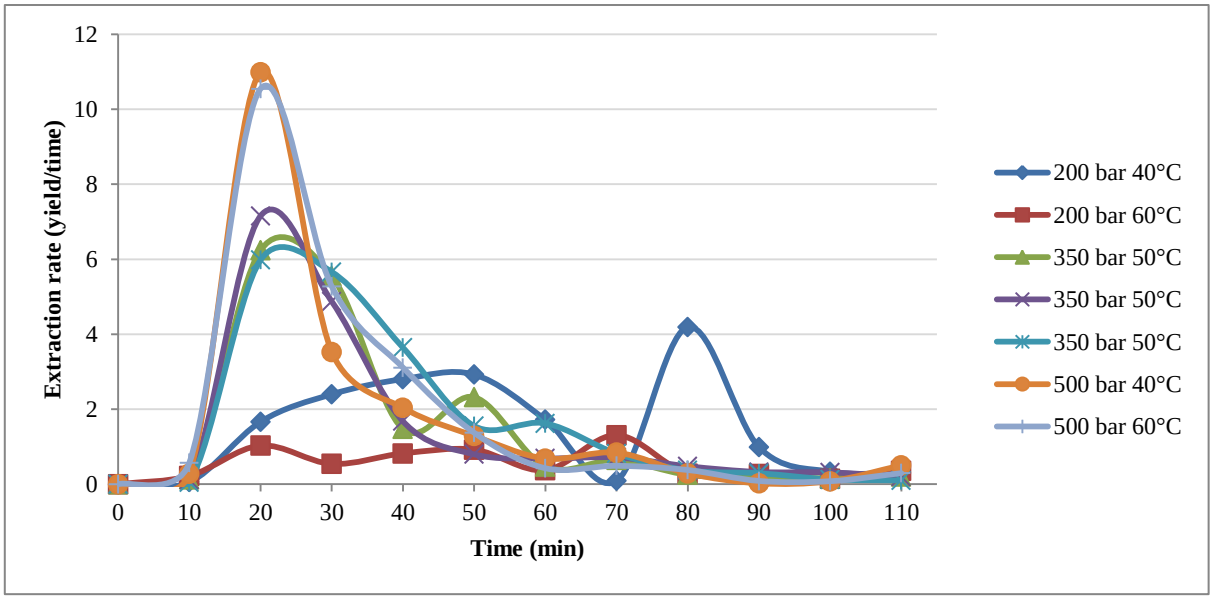

Fig. 6. Extraction rates for the SFE of bilberry seed oils extracted at different pressures and temperatures for 110 min.

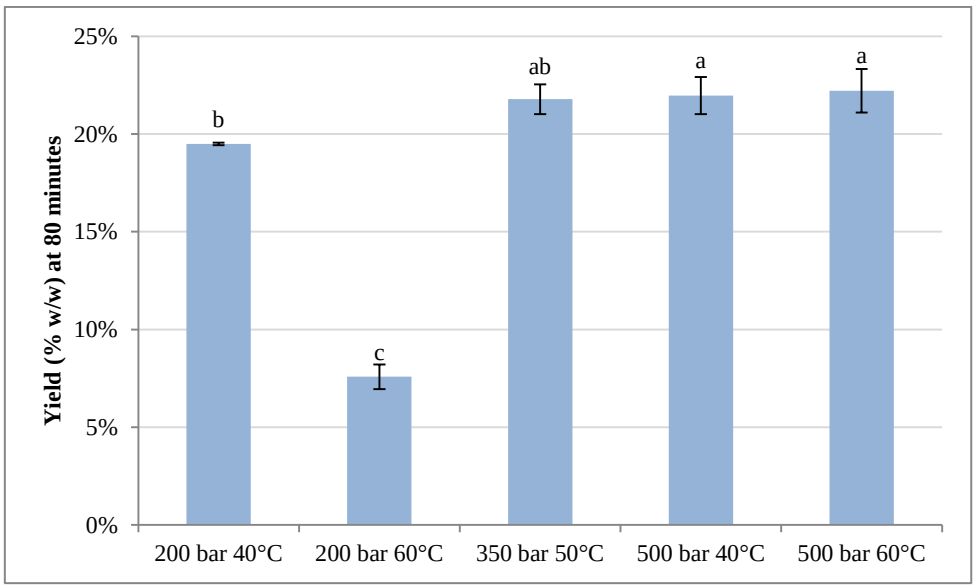

Fig. 7. Extraction yield (% w/w) of bilberry seed oils extracted at different pressures and temperatures for 80 min. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

An increase in the extraction yield after increasing the pressure at the same temperature has been previously reported using different raw materials, such as sea buckthorn pomace, blueberry, bilberry, passionfruit, and mucuna seeds [46, 49, 78-80]. However, many these studies have focused on optimisation of the extraction conditions for recovering specifically targeted compounds.

5.1.2 Effect of temperature

The effect of temperature on the extraction yield was only significant at 200 bar (**Fig. 7**). At this pressure, the increase in temperature led to a decrease in the extraction yield. The effect of temperature on extraction yield can be explained by the (opposite) effects exerted by temperature on oil solubility [45, 81]. At constant pressure, an increase in temperature decreases the solvent density, which increases the distance between the molecules, thereby decreasing the interactions between the oil and SC-CO_2, leading to a decrease in solubility [45]. According to de Melo and co-workers [69], most SFE studies use pressures in the range of 100–400 bar, giving solvent densities in the range of 200–900 Kg/m^3. They also reported that within the same temperature range of 40°–100°C, the density could vary by 70% at 100 bar and by only 20% at 500 bar. However, an increase in temperature also increases the vapour pressures of the solutes, improving the solubility of the oil in supercritical CO_2. When the temperature effect on vapour pressure more than compensates for the decrease in density, the solubility increases, resulting in a higher extraction yield. In mixtures of similar compounds, the inter-molecular interactions are alike, and the vapour pressure depends on the molecular weight [28]. However, in complex mixtures, the vapour pressure is more difficult to predict. **Figure 8** shows the effect of temperature on the extraction yields of cloudberry, black currant, and bilberry seeds, at constant pressure (350 bar) and 50°C and 80°C. The increase in temperature at constant pressure increased the extraction yields of cloudberry and black currant seeds, indicating that the effect of vapour pressure caused by the temperature of the SC-CO_2 dominated the effect on the solvent density. The extraction yield of bilberry seeds at 500 bar was not significantly affected by the temperature increase, suggesting that the magnitudes of the effects caused by temperature were similar and that the cross-over pressure was close to 350 bar. 'Cross-over pressure' is the pressure at which the temperature effect on the oil yield is the reverse, and therefore, an increase in temperature at this pressure results in similar extraction yields.

Table 2
Content of tocopherols (T) and tocotrienols (Tr) in cloudberry, black currant and bilberry seed oils

Samples	mg/100 g oil						mg/100 g dry seeds
	α-T	γ-T	δ-T	α-Tr	γ-Tr	Total	Total
Extraction time 80 min							
Bilberry 200 bar 40°C	ND	ND	11 ± 0	2.5 ± 0.1	57 ± 0	70 ± 1	14 ± 0
Bilberry 200 bar 60°C	ND	ND	25 ± 0	4.5 ± 0.2	100 ± 5	129 ± 5	10 ± 0
Bilberry 350 bar 50°C	ND	ND	11 ± 0	2.2 ± 0.1	49 ± 2	63 ± 2	14 ± 0
Bilberry 500 bar 40°C	ND	ND	11 ± 0	2.7 ± 0.0	53 ± 1	66 ± 0	15 ± 0
Bilberry 500 bar 60°C	ND	ND	10 ± 0	2.3 ± 0.1	46 ± 1	58 ± 1	13 ± 0
Extraction time 60 min							
Cloudberry 350 bar 50°C	38 ± 1	67 ± 3	4.9 ± 0.0	-	0.8 ± 0.0	111 ± 5	3.7 ± 0.2
Cloudberry 350 bar 80°C	40 ± 1	75 ± 2	4.9 ± 0.0	0.2 ± 0.0	1.1 ± 0.0	121 ± 5	8.2 ± 0.3
Black currant 350 bar 50°C	122 ± 1	103 ± 6	16. ± 1	0.3 ± 0.0	0.7 ± 0.0	242 ± 10	4.7 ± 0.2
Black currant 350 bar 80°C	93 ± 1	87 ± 0	134 ± 0	0.3 ± 0.0	2.4 ± 0.0	197 ± 2	12 ± 0
Bilberry 350 bar 50°C	18 ± 0	3.6 ± 0.2	0.3 ± 0.0	0.6 ± 0.0	36 ± 2	59 ± 3	7.8 ± 0.4
Bilberry 350 bar 80°C	26 ± 0	4.8 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	37 ± 2	69 ± 3	8.6 ± 0.4
Hexane extraction							
Cloudberry	40 ± 0	85 ± 2	5.5 ± 0.0	-	1.0 ± 0.1	132 ± 2	14 ± 0
Black currant	31 ± 1	70 ± 2	13 ± 0	-	0.1 ± 0.0	113 ± 4	11 ± 0
Bilberry	4.7 ± 0.2	1.6 ± 0.0	0.2 ± 0.0	-	11 ± 0	17 ± 1	3.2 ± 0.2

ND = Not determined

The temperature also influenced the recovery of antioxidants. **Table 2** shows that the extraction conditions influenced significantly the recovery of vitamin E (or tocopherols and tocotrienols) from bilberry seeds. The oil that was extracted at 200 bar and 60°C showed the highest recovery of vitamin E (129.2 mg/100 g oil). Bravi and co-workers [82] have also obtained higher concentrations of vitamin E at low pressure and high temperature during the extraction of grape seeds.

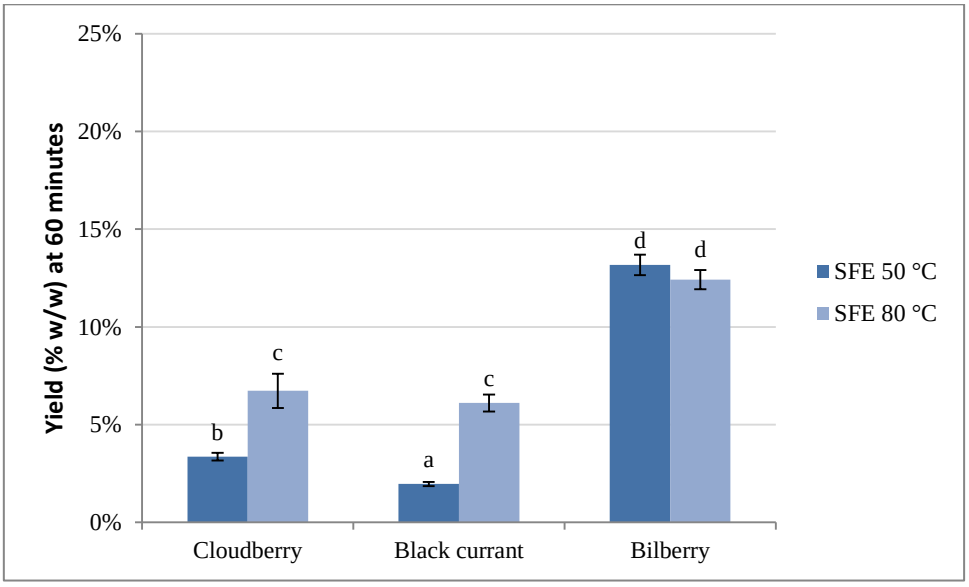

Fig. 8. Extraction yield (% w/w) of cloudberry, black currant and bilberry seed oils extracted at 350 bar and different temperatures (50 and 80°C). Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

The increase in temperature from 50°C to 80°C at 350 bar resulted in higher recovery of vitamin E and carotenoids (**Tables 2** and **3**, respectively) for the cloudberry and bilberry seeds, whereas the opposite effect was noted for black currant seeds. The extracts are mixtures of components, which could affect the solubilities of minor compounds, such as vitamin E. It is worth pointing out that the extraction conditions which gave higher recovery of antioxidants for a determined berry can differ from the extraction conditions which gave higher extraction yield. The current study was focused on identification of the extraction conditions with highest recovery of antioxidants. However, due to the low extraction yield obtained for the bilberry seed oil extracted at 200 bar and 60°C, for industrial applications it has to be taken into consideration if the purpose with the extraction is the transfer of valuable compounds or to obtain a high extraction yield of the oil from the by-products. Despite the extraction

conditions having an important effect in SFE, the opposite effect observed for black currant seeds is related to its matrix; these results will be further elaborated in this thesis.

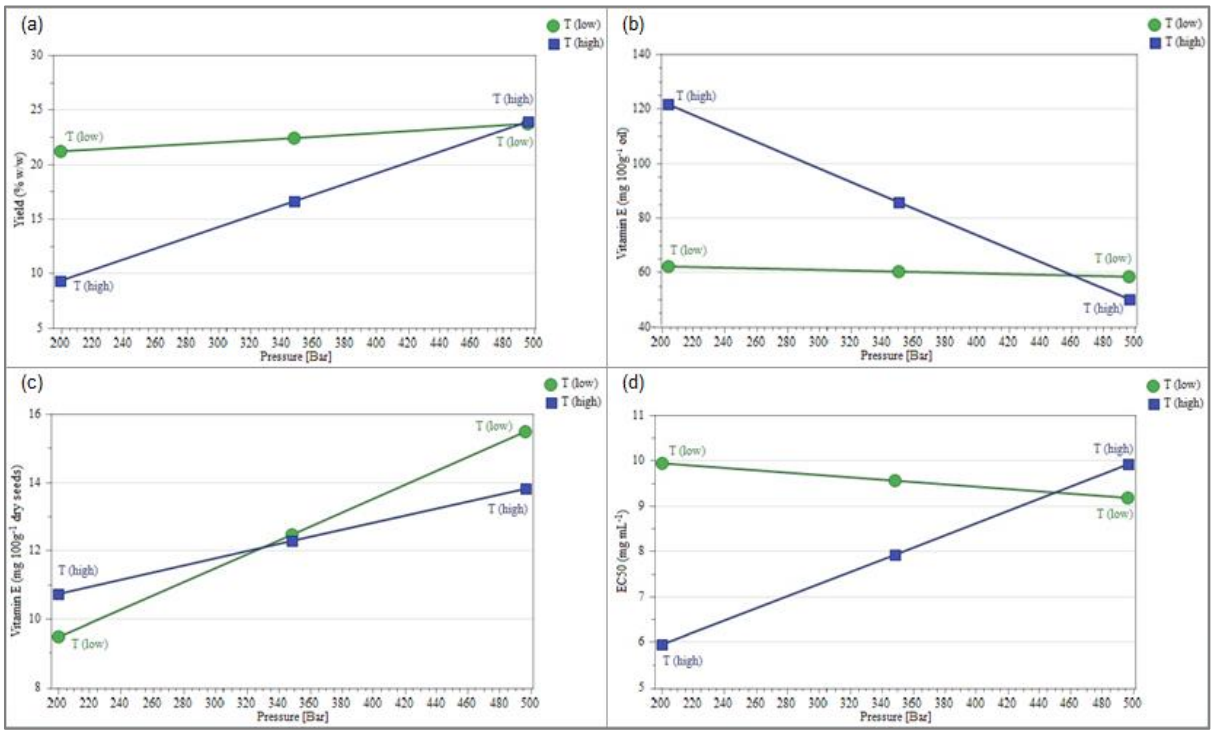

Fig. 9. Interaction plot for effect of temperature and pressure in extraction of bilberry seeds on: (a) yield; (b) vitamin E (mg/100 g of oil); (c) vitamin E (mg 100/g of dry seeds) and (d) EC50.

The effects of processing parameters and their possible interactions can be examined with screening designs, such as full factorial level [70]. Xu and co-workers used a 2^4 factorial design (two-level design in four factors) to evaluate the effects of pressure and temperature [83]. The interaction between pressure and temperature had a significant effect on the recovery of vitamin E and carotenoids from sea buckthorn. Da Porto and co-workers used a 3^3 full factorial design to evaluate the effects of pressure and temperature on the extraction yield of hemp seed and found that these parameters had significant relation [84]. The pressure and temperature conditions during extraction of bilberry seeds in the current study were based on a 2^2 full factorial design (**Fig. 4**). As shown in **Figure 9, s**ignificant interactions were found for both pressure and temperature ($p = 0.05$) during SFE of bilberry seeds. The plots indicate a mild interaction between pressure and temperature for extraction yield (**Fig. 9a**) and a strong interaction for vitamin E (g/100 g oil) (**Fig.**

9b), vitamin E (g/100 g dry seeds) (**Fig. 9c**), and EC_{50} (**Fig. 9d**).

5.1.3 Solubility behaviour in SC-CO_2

The solubility is strongly dependent upon the SC-CO_2 density and solute properties (e.g., molecular weight, polarity, and vapour pressure) [68]. The solvent masses of compressible solvents, such as SC-CO_2, are non-homogeneously distributed, creating regions with higher solvent density and consequently, increased solubility [61]. This phenomenon of local enhancement of density of SCFs around solute molecules, together with the resulting improvement in extractability is known as 'clustering' [61]. The solubility is also determined by the entropy change when the solute disperses in the solvent, and therefore, it is dependent upon the pressure, temperature, and system polarity [85]. Compounds with similar polarity are easily miscible, and a phrase that is commonly used to describe solubility in relation to polarity is: "like dissolves like" [57]. In the literature, the solubility data for the same compound vary considerably due to sample purity and factors related to the SFE system [28]. Plant oils are complex mixtures, and their solubilities in SC-CO_2 reflect inter-molecular interactions, since the different compounds can affect the solubility of one another [28]. Moreover, knowledge of the chemical and physical properties of minor compounds, such as vitamin E and carotenoids, is required to understand their solubility behaviours [28].

Vitamin E is fat-soluble and can be dissolved in relatively non-polar organic solvents, such as SC-CO_2. The polarity of vitamin E is mostly influenced by the number of methyl groups in the chromanol ring, and to a less extent it increases with the presence of unsaturated side-chains [30]. The melting point of vitamin E is low (e.g., 2.5°–3.5°C for α-tocopherol), indicating they are no solids in the matrix, which favours the extraction [28]. Carotenoids are also fat-soluble and their physical, chemical, and biological properties are mostly related to their elongated, symmetric chain structure, which can easily form crystals [86, 87]. The melting point of β-carotene is 183°C, considerably higher than that of α-tocopherol, and during SFE, β-carotene can undergo isomerisation, which affects its solubility, since its *cis*

isomers are more soluble than its *trans* isomers [28].

Table 3
Content of carotenoids in cloudberry, black currant and bilberry seed oils

Samples	mg/100 g oil				mg/100 g dry seeds
	Lutein	β-carotene	Carotene equivalents	Total	Total
SFE					
Cloudberry 350 bar 50°C	0.9 ± 0.0^{a}	4.3 ± 0.0^{g}	31 ± 0^{f}	37 ± 0^{f}	1.2 ± 0.0^{d}
Cloudberry 350 bar 80°C	0.2 ± 0.0^{a}	2.6 ± 0.0^{e}	35 ± 0^{g}	38 ± 0^{g}	2.5 ± 0.0^{e}
Black currant 350 bar 50°C	12 ± 0^{e}	-	4.8 ± 0.0^{b}	17 ± 0^{e}	0.3 ± 0.0^{a}
Black currant 350 bar 80°C	10 ± 0^{d}	1.4 ± 0.0^{d}	1.7 ± 0.0^{d}	13 ± 0^{c}	0.8 ± 0.0^{c}
Bilberry 350 bar 50°C	2.4 ± 0.0^{b}	0.1 ± 0.0^{a}	0.4 ± 0.0^{a}	2.8 ± 0.0^{a}	0.4 ± 0.0^{ab}
Bilberry 350 bar 80°C	3.2 ± 0.0^{c}	0.3 ± 0.0^{b}	0.6 ± 0.0^{a}	4.1 ± 0.0^{b}	0.5 ± 0.0^{b}
Hexane extraction					
Cloudberry	-	3.4 ± 0.0^{f}	54 ± 0^{h}	57 ± 0^{h}	5.9 ± 0.0^{h}
Black currant	31 ± 0^{f}	1.5 ± 0.0^{d}	5.6 ± 0.0^{e}	38 ± 0^{g}	3.8 ± 0.0^{g}
Bilberry	12 ± 0^{e}	0.9 ± 0.0^{c}	2.6 ± 0.0^{c}	16 ± 1^{d}	2.9 ± 0.1^{f}

Results are mean values ± standard deviation of triplicates and different letters within the same raw indicate significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

5.1.4 Structures of seeds

The matrix structure of oil seeds is often cellular, porous, and containing inter-cellular regions [88]. The porous structure favours SFE, since SCFs can easily penetrate porous materials due to their low viscosity and diffusivity [59]. Seed matrices often contain layers, which are composed of different cells. Some berry seeds, such as bilberry seeds, have thick-walled cells in the outermost layer, which can add extra resistance to mass transfer during SFE [17]. The spatial location of target compounds inside the cells is also important, since this determines how easily a targeted compound m can be extracted [28].

The vitamin E in plant cells is located in the cell membranes (**Fig. 1**) and forms complexes with lysophospholipids and PUFAs [23, 89]. SC-CO_2 can modify the membrane, increasing its fluidity through disruption of the order of the lipid hydrophobic chains, thereby destabilising the membranes and increasing permeability, resulting in enhancement of the extractability of compounds located in the cell membranes [90].

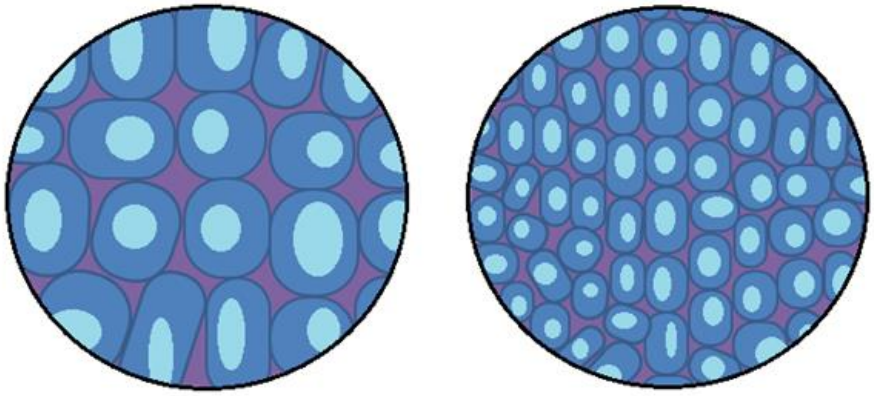

Fig. 10. Schematic illustration of seed particles showing cells of different sizes. The surface are of the particle with large cells (right) is smaller compared to the particles with small cells.

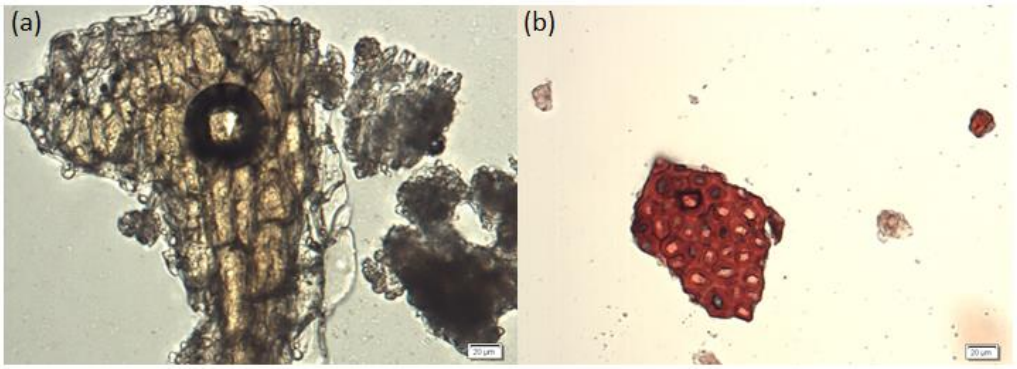

Fig. 11. Seeds of cloudberry (a) and black currant (b) dried and milled for 30 s. White arrow indicates oil droplet released after milling. Black arrows indicate disrupted cells.

The cell sizes of the seeds or other raw materials to be extracted can also influence the extraction efficiency. Milling of seeds with small cell sizes, such as black currant seeds, results in larger surface area, as compared to seeds with larger cell sizes, such as cloudberry and bilberry seeds. **Figure 10** illustrates the seed particles with different cell sizes; the panel on the right shows a larger surface area of the cell membranes because the cells are smaller. In the first period of extraction, the solute from broken cells diffuses into the fluid phase, and the seed type and cell structure can influence the efficiency of the process (Steps 3 and 4, **Fig. 2**) [55]. **Figure 11** shows the particles of cloudberry (**Fig. 11a**) and black currant seeds (**Fig. 11b**). The larger surface area of black currant seeds probably facilitated the extraction of vitamin E from these seeds, as compared to the cloudberry and bilberry seeds. The cloudberry and bilberry seeds had smaller surface areas of the cell membranes exposed

to the solvent and therefore, the mass transfer resistance of the solutes was higher during the extractions. As mentioned above, since extracts from plant matrices are complex, it is difficult to predict the solubility behaviours of the compounds in SC-CO_2.

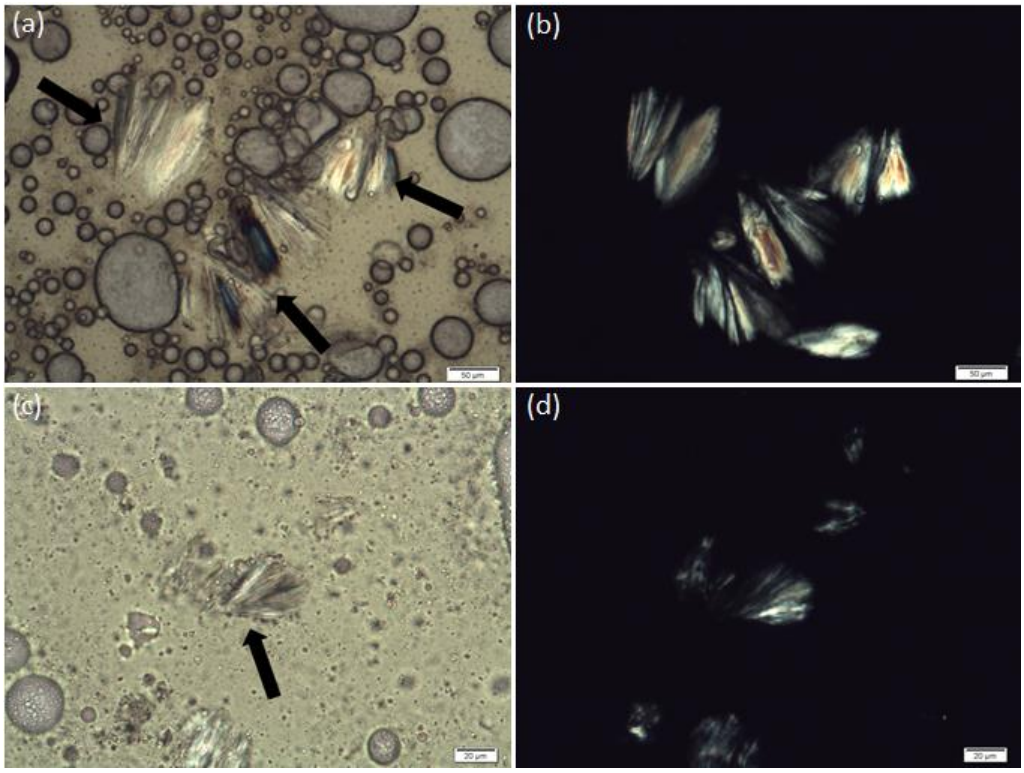

Fig. 12. Oil of cloudberry (a-b) and black currant seeds (c-d) extracted at 350 bar and 80°C. In (a) and (c), the carotenoids' crystals are indicated by the black arrows. In (b) and (d), the polarized images show carotenoids' crystals.

The carotenoids in fruits are normally accumulated in the chromoplasts (**Fig. 1**), with various forms and structures [91]. Chromoplasts are usually the end-stage of plastids, being produced when the fruit ripens, and their high concentrations of carotenoids gives the plants their yellow, orange, and red hues[92]. **Figure 12** shows LM images of the oils extracted from cloudberry (**Fig. 12a-b**) and black currant seeds (**Fig. 12, c-d**), revealing the presence of what are believed to be carotenoids crystals. Cao and co-workers [93] confirmed the nature of these crystals using polarisation microscopy (**Fig. 12, b-d**), and the reddish colour (more clearly evident in **Fig. 12b**) is associated with the accumulation of β-carotene. The cloudberry seed oil had a higher number of crystals, in agreement with the high concentrations of carotenoids detected in the oil (**Table 3**). Several peaks for carotene equivalents

were identified as all-*trans* carotenes, which are linear, rigid molecules that can easily form crystals that fit into sub-cellular structures [87]. The crystals observed in the black currant seed oil were considerably smaller than those in the cloudberry and bilberry seed oils. The differences in sizes and shapes suggest variations in the compositions of the carotenoids in the crystals. Moreover, these differences in the crystals could have influenced their solubility in SC-CO_2 and thus, their extractability during SFE. Further investigations into the extractability of the crystals and the physical changes that may occur in the crystal structures during SFE are warranted.

Different aspects of the matrices should be considered before extraction, such as cell size, shape, and hardness, presence of seed shells, localisation and levels of extractable solutes in the matrix, and the species of the matrices [28, 51, 68]. After evaluating these aspects, it is also necessary to relate them to the four stages of SFE (**Fig. 2**), so as to increase extraction efficiency. These steps happen inside the matrix, especially Steps 3 and 4, which comprise the dissolution of the extract in SC-CO_2 and the diffusion of the mixture (SC-CO_2 + extract) out of the matrix, respectively, and they can be directly influenced by the different aspects of the matrices.

5.1.5 Effects of pre-treatment on extraction

The cloudberry, black currant, and bilberry seeds were dried and milled before the extractions, breaking the seeds. The pre-treatments (drying and milling) broke the seeds in small particles, also breaking the cells located in the particle surface, immediately releasing a fraction of the oil that could be easily extracted. After milling, the seed particles clustered together and stuck to the mill's blades, especially after the milling of bilberry seeds, suggesting a higher level of oil in these seeds and that the oil is released when the cells are broken. Interestingly, the black currant seeds were initially dark but became pink in colour after milling, given that they have a different colour in the interior part of the seeds, and indicating that the structure of the black currant seed is heterogeneous. In addition, the cloudberry seeds had hard shells, which were immediately detached from the seeds during milling.

The disrupted cells are indicated by arrows in **Figure 11**, **a** and **b**. The large cells of cloudberry seeds may facilitate the

extraction in the inner part of the broken cells (**Fig. 11a**). The small cells of black currant seeds (**Fig. 11b**) had a larger membrane surface than the cloudberry seeds, favouring the extraction of vitamin E. Longer milling times could have been used to break the seeds into smaller particle sizes, increasing the mass transfer, and providing higher extraction yields. However, smaller particle sizes could have emerged in uneven extractions due to a channelling effect inside the extraction vessel [49, 52]. The channelling effect is also associated with high flow rates, which can compact small particle sizes and cause them to flow around the channels inside the vessel. This effect reduces the contact between the SC-CO_2 and the sample, diverting the normal flow through the pores within the matrix, which reduces the extractability [45]. The occurrence of channelling can be verified by a decrease in the extraction yield and by an uneven colour distribution in the matrix after extraction, indicating that the oil has only been extracted from certain regions of the matrix [45, 49]. Therefore, it is important to evaluate different milling times and the resulting particle sizes when determining the extraction conditions. In the current study, no channelling effect was detected.

According to Johansson and co-workers [21], the average weights of the cloudberry, black currant, and bilberry seeds were 760, 90, and 10 mg, respectively. Differences in the weights and sizes of seeds can produce heterogeneous particles after milling, with different size ranges. Del Valle and Uquiche [94] found, for rosehip seeds, that different size ranges of the milled seeds resulted in fractions with different oil contents. They also found that large fractions of seed particles contained mostly fragments of the seed structures, which were devoid of oil, while the small particles contained mainly germ fragments with a high oil content. **Figure 11b** is shows the different particle sizes of the black currant seeds; the largest particle (indicated with a black arrow) is composed of cells with extractable solutes, while the small particles are probably from parts of the seeds that are devoid of oil. The mechanical behaviour of the fraction was probably determined by the differences in hardness of the seed parts, which led to different particle sizes for different parts of the seeds. As mentioned above, target compounds, such as vitamin E and carotenoids, are accumulated in specific cell structures. The different size fractions could also lead to the separation of seed particles

with higher content of vitamin E and/or carotenoids. Thus, separating seed particles according to size range could be a useful strategy for selecting which seed fragments will be extracted, thereby increasing the extraction efficiency.

5.2 Sustainability aspects of using berry seed oils obtained by SFE

SC-CO_2 is a suitable solvent for use in the food industry as it is non-toxic and non-flammable, and it can be easily removed from the extracts [45]. Moreover, SFE extracts are solvent-free, since the solvent is separated from the extract at the end of the SFE process. The SC-CO_2 can also be recycled, saving energy and lowering costs [81]. These advantages make SFE an environmentally friendly technology that is an ideal replacement for the conventional solvent-based extraction methods.

Solvent extractions have been associated with several environmental disadvantages, such as the unpleasant odour of hexane vapour, oil spillage in water bodies, impoundment of dirty and oily water, and the dust released into the air [95]. Despite the well-known disadvantages of using hexane as a solvent, a better comparison of the extraction methods can be made with a life cycle analysis, which quantifies the environmental impacts associated with a product, process or activity [96]. Studies comparing SFE with hexane extraction have shown that the SC-CO_2 solvent has no significant impact, although SFE has a strong environmental disadvantage in terms of electricity consumption [97]. However, most of the electricity consumption, which is used for heating and pumping, can be reduced when scaling-up SFE [97, 98].

Figure 13 shows the yields of cloudberry, black currant, and bilberry seed oils extracted by SFE under a constant pressure (350 bar) and at 50°C and 80°C, and using hexane extraction. Even though hexane extraction confers higher extraction yields and hexane is considered to be a better solvent for recovering carotenoids (**Table 3**), extraction with organic solvents may not be acceptable for food applications [96]. Furthermore, the oils obtained by solvent extraction need to be refined to remove unwanted impurities. Oil refining consists of several steps in which the oil is exposed to high temperatures and air contact, resulting in degradation of the antioxidants present

in the oil [99]. After refining, the final product still contains solvent residues, while oils extracted by SFE are free of solvents and therefore, are suitable for food applications [84]. Thus, berry seed oils recovered by SFE from by-products have the potential to be used in the food industry, minimising the side-stream during processing.

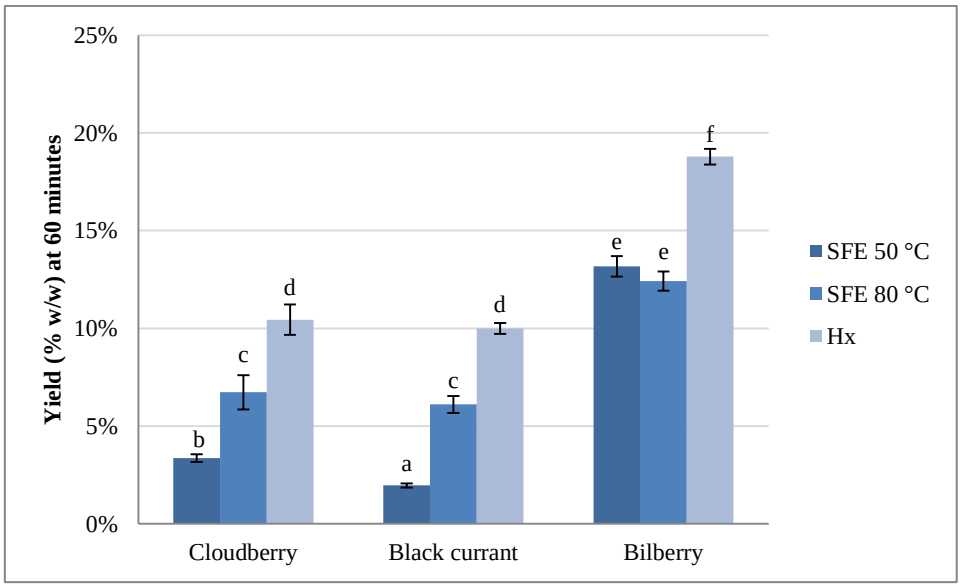

Fig. 13. Extraction yields (% w/w) of cloudberry, black currant and bilberry seed oils extracted by SFE at different temperatures and by hexane extraction. Results are mean values ± standard deviation of triplicate extractions. Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

5.3 Utilisation of extracts from berry seeds obtained by SFE

The utilisation of berry seed oils obtained by SFE is considered based on the contents of PUFAs, vitamin E, and carotenoids, as well as the possibilities to use these oils.

5.3.1 Chemical composition of berry seed oils

Berry seed oils are rich in lipids and in general, have high contents of PUFAs. **Table 4** shows the fatty acid compositions and the ω6/ω3 ratios of the berry seed oils extracted by SFE under different conditions and using hexane extraction. All the oils analysed had high percentages of PUFAs (66.8% to 75.9%) and the major fatty acids were linoleic (18:2 ω6) and α-linoleic acid (18:3 ω3). These results are in agreement with previous findings, which reported high contents of essential FAs, such as linoleic (18:2 ω6) and α-linolenic (18:3 ω3)

acids for cloudberry, black currant, and bilberry seed oils [5, 48, 100]. In addition, the black currant seed oil contained stearidonic acid (18:4 ω3) and high levels of γ-linolenic acid (18:3 ω6). A similar FA composition for black currant seeds has been previously reported [5, 101]. Stearidonic acid is an intermediate metabolite during the conversion of α-linolenic acid and eicosapentaenoic (20:5 ω3) or docosahexaenoic (22:6 ω3) acids. Moreover, stearidonic acid is highly unsaturated and therefore, is implicated in improvements in inflammatory and in CVD [102]. The γ-linolenic acid attenuates inflammatory responses and α-linoleic acid is associated with health effects, such as decreased risk of CVD [101, 103]. Therefore, black currant seed oil can be a good source of these PUFAs, especially for consumers who aim to reduce their risk of CVD.

The vitamin E profiles (**Table 2**) of bilberry seed oils extracted at 350 bar and 50°C for different extraction times (80 min and 60 min) were different, particularly in terms of their δ-T contents. The levels of δ-T in the bilberry seed oils extracted for 80 min were considerably higher (11.0 ± 04 mg/100 g oil) than the oils extracted for 60 min (0.3 ± 0.0 mg/100 g oil). This suggests that the additional extraction time may have facilitated better dissolution of the δ-T in SC-CO_2. Previous studies [62, 104] have shown that the concentration of δ-T increases under stress conditions, which may also have contributed to the different contents of δ-T, since the bilberry seeds used in the current study were grown in different locations [62]. Furthermore, the bilberry seeds belonged to different cultivars, which can also influence the composition. The concentrations of vitamin E in the bilberry seed oils extracted by SFE were higher (give the level) than those reported in the literature (40 mg/100 g oil), and only α-Tr and γ-Tr were detected in the other study [5].

The cloudberry and black currant seed oils had considerably higher concentrations of vitamin E than the bilberry seed oils (**Table 2**). Vitamin E biosynthesis can change during plant development and with different stresses during cultivation [62]. Another important aspect that should be considered is the classification of berries. All the berries investigated in this study belong to the class Magnoliatae. However, bilberry belongs to the subclass Dilleniidae, while cloudberry and black currant belong to the subclass Rosidae, order Rosanae [21]. The taxonomic proximity of cloudberry and black currant

Table 4
Fatty acid composition of cloudberry, black currant and bilberry seed oils

Samples	Fatty acids % (w/w)										ω6/ω3 ratio
	16:0	18:0	18:1 n-9	18:1 n-7	18:2 n-6	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	
Extraction time 80 min											
Bilberry 200 bar 40°C	5.5 ± 0.1	1.5 ± 0.1	23 ± 0		34 ± 0	32 ± 0					0.93 ± 0.00
Bilberry 200 bar 60°C	6.1 ± 0.1	1.4 ± 0.1	23 ± 0		34 ± 0	36 ± 0					0.95 ± 0.01
Bilberry 350 bar 50°C	5.3 ± 0.1	1.5 ± 0.0	23 ± 0		34 ± 0	36 ± 0					0.93 ± 0.00
Bilberry 500 bar 40°C	5.3 ± 0.1	1.5 ± 0.0	23 ± 0		34 ± 0	36 ± 0					0.93 ± 0.00
Bilberry 500 bar 60°C	5.3 ± 0.1	1.6 ± 0.1	23 ± 0		34 ± 0	36 ± 0					0.93 ± 0.00
Extraction time 60 min											
Cloudberry 350 bar 50°C	3.7 ± 0.1	1.8 ± 0.0	16 ± 0	1.0 ± 0.0	42 ± 0	32 ± 0	1.5 ± 0.0		1.1 ± 0.0	0.9 ± 0.0	1.33 ± 0.00
Cloudberry 350 bar 80°C	3.4 ± 0.0	1.8 ± 0.0	17 ± 0	1.0 ± 0.0	41 ± 0	34 ± 0			1.2 ± 0.9	0.9 ± 0.0	1.22 ± 0.00
Black currant 350 bar 50°C	7.9 ± 0.0	2.1 ± 0.0	12 ± 0	1.0 ± 0.0	43 ± 0	16 ± 0	13 ± 0	3.5 ± 0.1		0.8 ± 0.0	2.81 ± 0.01
Black currant 350 bar 80°C	7.7 ± 0.1	2.1 ± 0.1	13 ± 0	1.1 ± 0.0	42 ± 0	18 ± 0	13 ± 0	3.2 ± 0.2		0.7 ± 0.0	2.65 ± 0.06
Bilberry 350 bar 50°C	5.9 ± 0.1	1.6 ± 0.0	24 ± 1	1.0 ± 0.1	33 ± 2	35 ± 1					0.94 ± 0.06
Bilberry 350 bar 80°C	6.1 ± 0.5	1.8 ± 0.5	24 ± 0	1.1 ± 0.1	33 ± 1	34 ± 0					0.99 ± 0.00
Hexane extraction											
Cloudberry	3.4 ± 0.0	1.9 ± 0.0	17 ± 0	1.0 ± 0.0	41 ± 0	34 ± 0			1.2 ± 0.0	1.0 ± 0.0	1.20 ± 0.01
Black currant	7.7 ± 0.6	2.4 ± 0.0	13 ± 0	1.1 ± 0.1	44 ± 0	15 ± 1	13 ± 0	3.6 ± 0.4		0.8 ± 0.0	3.16 ± 0.18
Bilberry	6.1 ± 0.2	1.7 ± 0.1	24 ± 0	1.1 ± 0.0	33 ± 1	34 ± 0					0.97 ± 0.02

might explain some of the similarities in their chemical compositions. The concentration of cloudberry seed oil (111–132 mg/100 g oil) was lower than that reported in the literature (260–270 mg/100 g oil). In contrast, the concentration of black currant seed oil (242–113 mg/100 g oil) was substantially higher than that reported by other authors (100 mg/100 g oil) [5, 105].

Carotenoids are important micronutrients that have been linked to several health benefits. Black currant and bilberry seed oils have lutein as their major carotenoid (30.8 and 12.1 mg/100 g oil, respectively). Information about lutein or other carotenoids in berry seed oils are still scarce. Previous studies only reported the lutein concentration of the whole berry: 0.21 and 0.23–0.25 mg/100 g of fresh black currant and bilberry, respectively [100, 106]. As is the case for other carotenoids, lutein is a pigment that is synthesised in plants. Lutein has antioxidant activity and is one of the few carotenoids present in the eye lens, retina, and macular pigment [107].

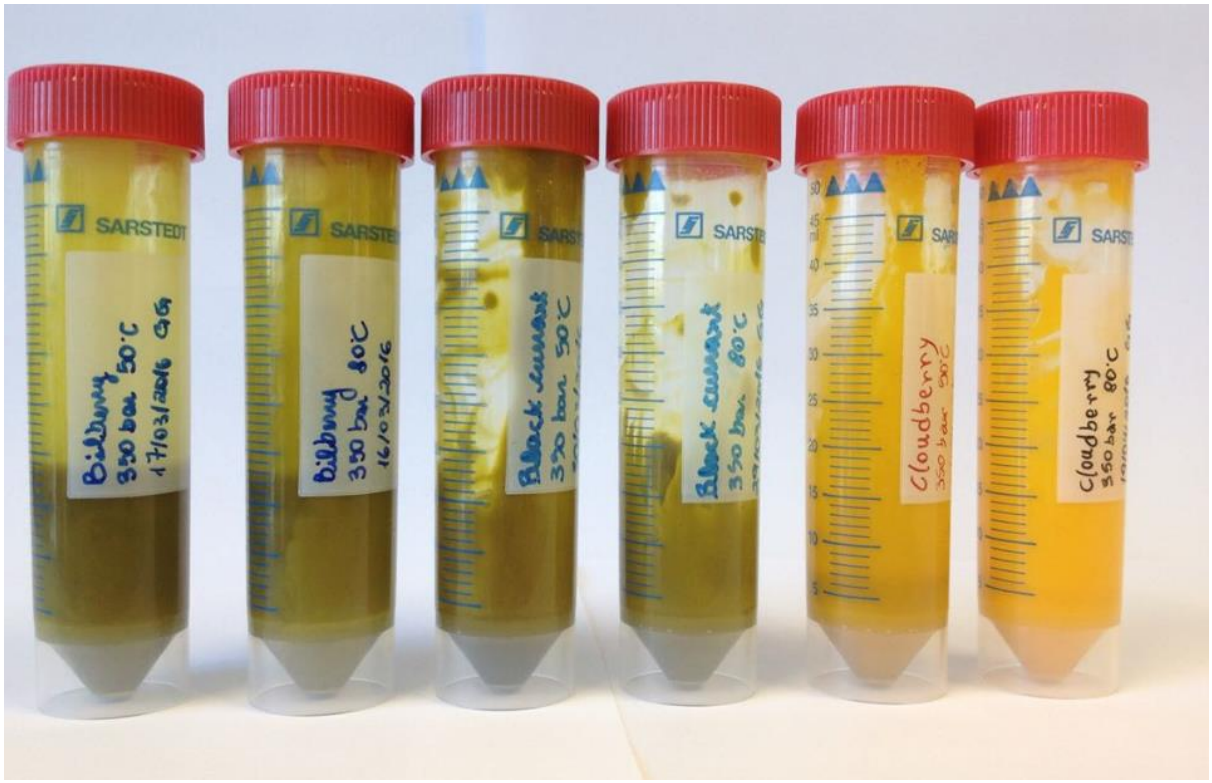

Fig. 14. Bilberry, black currant and cloudberry seed oils, extracted at 350 and different temperatures (50 and 80°C).

Cloudberry has β-carotene as the major carotenoid and also has the highest concentrations of carotene equivalents. The

concentrations of carotenoids were slightly lower than those reported by Manninen and co-workers for the total amount of carotenoids (64 mg/100 g oil) in cloudberry seed oils extracted at 300 bar and 40°C [105]. **Figure 14** shows the oils extracted from the cloudberry, black currant, and bilberry seeds. The high concentration of carotenes is reflected in the intense orange colour of the cloudberry seed oils. While black currant seed oils also contain high concentrations of carotenoids, they are greenish in colour, similar to the bilberry seed oils. Compounds other than carotenoids can also add colour to the oils. In the carotenoid analysis, several peaks were identified as chlorophylls using the spectra library. Chlorophylls are green pigments, and this might explain the colours of the black currant and bilberry seed oils [108]. The health compounds present in these berry seed oils make them attractive alternatives for conventional oils.

5.3.2 Possibilities of using berry seed oils

Western diets are often deficient in ω3 and contain excessive levels of ω6, resulting in high ω6/ω3 ratios [109, 110]. Fish oils are commonly used to improve ω6/ω3 ratios due to their high content of ω3 PUFAs. However, their long-chain PUFAs are very susceptible to lipid oxidation [111]. Different nano- and micro-encapsulation techniques, such as emulsification and spray-drying, have been used in an attempt to protect the sensitive compounds against lipid oxidation [112]. Encapsulation can also mask the fishy odour and taste, preventing them from affecting the sensorial quality of the food and enabling delivery of compounds to specific sites within the body [113, 114]. Natural antioxidants have been incorporated into fish oil emulsions and microcapsules to prevent lipid oxidation [115-117]. Moreover, fish oils often come contaminated with pollutants (e.g., organochlorine pesticides and dibenzofurans) that bio-accumulate in fish, especially fish at the top of the food chain [118]. Cloudberry and bilberry seed oils have the advantages of having a content of vitamin E that naturally protects their PUFAs, and a lack of pollutants. However, encapsulation of berry seed oils would still be necessary, both for the delivery of compounds and to mask the strong colours of these oils, which could limit their use.

The recommended ω6/ω3 ratio from the health perspective is in the range of 1–2 [109, 110]. The cloudberry and bilberry seed oils had ω6/ω3 ratios (1.0–1.3) within this range. Chia seed oil is a current option for vegan supplement and its ω6/ω3 ratio is approximately 0.4 [119, 120]. Despite the ω6/ω3 ratio of chia seed oil being lower than those of berry seed oils, berry seed oils contain higher levels of vitamin E than chia and the wide availability of berry seeds in Sweden makes cloudberry and bilberry seeds interesting options for encapsulation. However, there are differences between the PUFAs from plants and fish. In plants, the most common FAs are linoleic and α-linolenic acids, which are essential FAs due to not be synthesized by the human body [103, 109]. The arachidonic, eicosapentaenoic and docosahexaenoic acids found in fish oil can be metabolized by the human body from linoleic and α-linolenic acids, but in limited amounts [103]. Despite the functional and biochemical differences between these two classes of PUFAs, both classes are important for metabolism [103].

5.3.3 Quality and stability of the oils

All bilberry seed oils have γ-Tr as the major constituent. In general, tocopherols are considered to be stronger antioxidants than tocotrienols. It has previously been shown that the order of antioxidant activities for both tocopherols and tocotrienols is α > β > γ > δ [30]. However, tocotrienols in oil and fat systems have been reported as having higher antioxidant activities than their corresponding tocopherols, especially γ-Tr [121]. Thus, both the content and the composition of the tocol constituents, with γ-tocotrienol as the major constituent, may contribute to the antioxidant activities of bilberry seed oils. Moreover, compared to tocopherols, γ-tocotrienol is reported to have superior neuroprotective, anti-cancer, and radioprotective properties [122]. Even though the cloudberries and black currants had lower levels of γ-Tr, they had high levels of α-T and γ-T (**Table 2**), which also have strong antioxidant activities. A mixture of tocols, such as α-T + γ-T or α-Tr + γ-Tr, has been reported to have synergistic effects, whereas a mixture of α-T + β-T or δ-T, and α-Tr + β-Tr or δ-Tr showed antagonistic effects [123]. It is important to know the composition and concentrations of the different tocols in edible oils. Since the individual antioxidants influence each other’s antioxidant

activities, it is difficult to predict the final outcome for oils that contain a mix of tocols and carotenoids.

Synergistic interactions between vitamin E and carotenoids have also been reported in literature [124, 125]. The antioxidant activities of carotenoids are not only restricted to scavenging activities; these compounds also act as quenchers, by transferring and dissipating the singlet oxygen energy [126, 127]. The synergistic effect can be explained by the ability of carotenoids to transfer electrons to the α-tocopheroxyl radical to regenerate tocopherol, which can continue to act as an antioxidant [125]. Variations in the contents of one or more antioxidant can affect the synergistic effect, and therefore, the overall antioxidant activity.

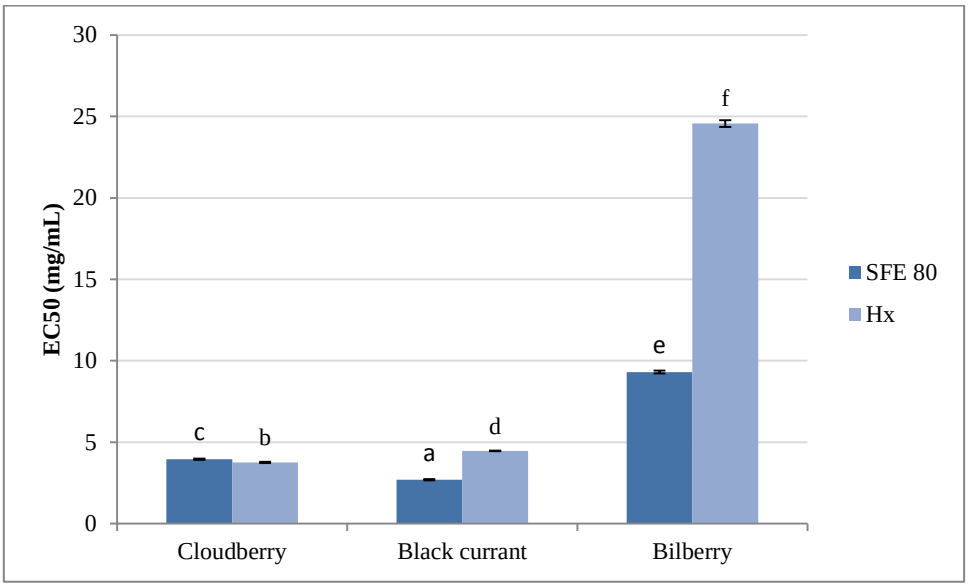

Fig. 15. EC_{50} of cloudberry, black currant and bilberry seed oils extracted at 350 bar and different temperatures (50 and 80°C). Different letters above error bars denote significant difference ($p < 0.05$) according to ANOVA followed by Tukey's post-hoc test.

The diversity of natural antioxidants present in plant oils makes it difficult to clarify the antioxidant activity of each compound and the effects of the compounds on each other. Antioxidant assays allow evaluations of the antioxidant effects resulting from the mix. The scavenger activity of a compound can be evaluated by colorimetric methods, such as the DPPH assay, which measures scavenger or reduction reactions between an antioxidant and a probe (oxidising agent) [33]. The EC_{50} value represents the concentration of extract that reduces the DPPH absorbance by 50%, whereby lower values indicate that the extracts have stronger antioxidant activities [75]. **Figure**

15 shows the EC_{50} values of the cloudberry, black currant, and bilberry seed oils extracted by SFE and those extracted by hexane. The bilberry seed oil extracted using hexane gave considerably higher EC_{50} values. The cloudberry and black currant seed oils had low EC_{50} values, reflecting strong antioxidant activities and indicating they can probably protect PUFAs against lipid oxidation. According to the IUPAC Technical Report [33], α-T and α-Tr are the most effective tocols in the DPPH assay. The bilberry seed oil extracted using hexane had the lowest concentration of α-T and lacked α-Tr, while the black currant seed oil extracted at 350 bar and 50°C had the highest concentration of α-T, suggesting that the high content of α-T might have resulted in low EC_{50} values. Comparison of different DPPH methods are limited, since several DPPH methods have been reported to use different initial concentrations of DPPH solutions, as well as different DPPH solution:extract solution ratios [128]. Kagliwal and co-workers [75] used a similar DPPH method for sea buckthorn seed oils and found EC_{50} values that were substantially higher (56.41 to 92.01) than the EC_{50} values for the cloudberry, black currant, and bilberry seeds, indicating that sea buckthorn seed oils have lower antioxidant activities.

The high concentrations of antioxidants, along with the associated strong antioxidant activities and synergistic effects, together with the FA profile suggest that cloudberry, black currant, and bilberry seed oils are valuable sources of antioxidants.

6 Conclusions

Supercritical fluid extractions of oils from cloudberry, black currant, and bilberry seeds were carried out at different extraction conditions, and the impacts of these conditions on the yields and chemical compositions of the extracts were evaluated. The main conclusions drawn from this thesis are as follows:

- The matrix plays a crucial role in the extraction. The variabilities in seed and cell sizes of different matrices lead to differences in the particle sizes after milling. Black currants have small seeds and seed cells, which means that they have smaller particles with larger surface area, favouring oil release and the extraction of vitamin E, which is located in the membranes.
- The increase in pressure at the same temperature increases the SC-CO_2 density, resulting in higher extraction yields.
- The increase in temperature at the same pressure has variable effects on the berries, although it tends to promote higher extraction yields at high pressures, due to the increase in vapour pressure of the solutes caused by the temperature increase. Increasing the extraction temperature at 350 bar has a positive effect on the recovery of vitamin E and carotenoids from cloudberry and bilberry seeds. The opposite effect is noted for black currant seeds, attributable to differences in the cell and particle sizes.
- Black currant seed oil contains high levels of PUFAs (75.1%–75.9%), and is the only berry seed oil of those tested that contains stearidonic and γ-linolenic acid. These findings indicate that black currant seed oil is a good source of PUFAs.
- The FA profiles of the cloudberry and bilberry seed oils, combined with their low ω6/ω3 ratios (1.0–1.3) suggest these oils as interesting options for encapsulation. Moreover, the presence of vitamin E in the seed oils suggests protection for PUFAs against lipid oxidation.

7 Future work

The differences in the microstructures of the analysed berries suggest that the extraction of target compounds could be optimized based on the location of these compounds. In future work, the effect of different pre-treatments, e.g. different milling time, will be done to optimize the recovery of antioxidants.

The results also show that the oils extracted from cloudberry, black currant, and bilberry seeds by SFE have strong potential to be used as ingredients in food formulations.

In future work, polar and non-polar extracts of bilberry by-products will be used to develop a non-thermal emulsification method (o/w/o and o/w). This will be based on exploiting the fact that bilberry seed oil is located in the inner phase surrounded by an aqueous phase of anthocyanins stabilised by whey protein isolate. The rheological properties and stability during storage will also be investigated.

It would be interesting to perform additional experiments to elucidate the microstructures of seeds before and after extraction. Although there have been many studies on SFE of berry seeds, few have investigated the seed microstructures in order to understand their influence on SFE.

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